

SCIENTIFIC PROCEEDINGS.

VOLUME XXIII.

MARCH, 1926.

No. 6.

New York Meeting

*University and Bellevue Hospital Medical College,
March 17, 1926.*

2992

**Puffer fish oil; a very potent antirachitic; its elaboration by fish
deprived of sunlight.**

ALFRED F. HESS and MILDRED WEINSTOCK.

*[From the Department of Pathology, College of Physicians and
Surgeons, Columbia University, New York City.]*

The most potent source of the antirachitic factor is fish oil, that from the liver of the cod having been found to be most active. In the course of an assay of various foods, etc., it was ascertained that the liver of the puffer fish (*Spheroïdes maculatus*) not only contains a large quantity of oil, but that this oil has exceptional antirachitic power. The oil was prepared from the liver by means of steam, by a method similar to that which is made use of in extracting oil from the livers of the cod. On testing this puffer fish oil biologically, it was found to be at least 15 times as potent as the most active specimens of cod liver oil. Such was the case in preventive tests in which the Sherman-Pappenheimer diet was fed, as well as in curative tests in which the McCollum diet was employed as a basal ration. No doubt if the stearine had been removed, the activity of the oil would have been proportionately increased.

No investigations have been carried out to ascertain whether the remarkable antirachitic potency of fish oil is due to the diet of the fish or to the intensity of the solar rays to which they are

subjected in nature. In order to study this question, oil was prepared from the livers of puffer fish which had been maintained in an aquarium in a tank for a period of 3 months. The fish were of the same batch which had been previously tested. During this period of captivity no active ultra-violet radiations had access to the fish, as the windows of the building were closed at all times. In spite of the fact that the fish were deprived for 3 months of activating rays, quantitative tests showed that their livers contained oil equal in potency to that which had been prepared shortly after they had been removed from the sea. The diet of these fish consisted largely of herring, which is very rich in antirachitic substance.

That the oil in the livers had been elaborated during the period of observation and was not merely residual oil which had been in the liver previous to captivity, is demonstrated by the fact that the livers became exceptionally rich in oil during the time the fish were deprived of ultra-violet rays. The average amount of oil which could be extracted initially was less than 5 per cent, whereas three months later 55 per cent of oil was extracted.

From this experiment we infer that the antirachitic factor can be elaborated in undiminished potency by fish deprived of sunlight for long periods, and that this characteristic quality of fish liver oil is dependent on the diet of the fish, rather than on the action of ultra-violet radiations.

2993

Action of ultra violet light upon bacteriophage and filterable viruses.

EARL B. MCKINLEY, RUDOLPH FISHER* and MARGARET HOLDEN.

[From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University, New York City.]

The theories concerning the nature of bacteriophage are well known. D'Herelle¹ has maintained from the very beginning that

* During tenure of Fellowship in Medicine, National Research Council.

¹ d'Herelle, F., *The Bacteriophage*, Fr. Edition, Oct., 1921; Eng. Edition, March, 1922.

the bacteriophage is a living ultramicroscopic virus. In this contention he has been opposed chiefly by the Bordet school, who consider the bacteriophage an inanimate agent which is developed by the microbe as a result of a reaction between the invading organism and the tissue cells of the host; that by the hereditary vitiation of the organism a lytic principle is developed.

Recently one of us has brought forward further evidence² in support of the Bordet conception and the lytic principle we have employed in these experiments is fully described in these publications.

This principle is markedly lytic for a strain of *B. coli* which we have designated *B. coli* "D" because of its diffuse growth in broth as contrasted with another strain which agglutinates spontaneously. Various dilutions of this bacteriophage were made in measured quantities of beef extract broth of pH 7.6. The materials to be exposed to the ultra violet light were placed in quartz test tubes which were obtained from the General Electric Co. To some of the tubes three to ten drops of a twenty-four hour broth culture of *B. coli* "D" were added. Other tubes contained both culture and lytic principle in amounts varying from three to ten drops. All of the tubes contained exactly 5 cc. of broth. The experiments were further controlled by exposing the medium alone to the action of ultra violet light before organisms or lytic principle were added. (Table 1.)

TABLE 1.

Tube containing 5 cc. broth	24-hour culture <i>B. coli</i> "D"	Lytic Principle	Exposure to ultra violet light at 1 ft. distance	Test for Lytic Principle.		
				Exp. 1.	Exp. 2.	Exp. 3.
I	3 drops	0 drops	40 min	—	—	—
II	6 "	0 "	40 "	—	—	—
III	10 "	0 "	40 "	—	—	—
IV	3 "	3 "	40 "	—	—	—
V	6 "	6 "	40 "	—	—	—
VI	10 "	10 "	40 "	—	+	—
VII	*3 "	10 "	40 "	—	—	—
VII	†3 "	10 "	40 "	—	+	+
VIII	3 "	0 "	40 "	—	—	—
IX	3 "	3 "	0 "	+	+	+
X	†3 "	†3 "	40 "	+	+	+

² McKinley, E. B., *C. R. de la Soc. de Biol.*, 1925, xei, 1050-2.

*Introduced 18 hours after irradiation.

†Introduced immediately after irradiation.

All tubes, except the lytic principle control tube, were exposed to ultra violet light radiation as projected from the Alpine Sun Lamp,[†] of 4.5 amperes at a distance of one foot for forty minutes. The apparatus was allowed to heat for five minutes before the exposures were begun. The quartz tubes were laid in open Petri dishes at an angle of about 5 degrees and their contents agitated every eight minutes during the exposure. During exposure the temperature did not exceed 30 degrees C. After the exposure to ultra-violet light the tubes were incubated at 37.5 degrees C. for twenty-four hours. The cultures were then tested for the presence or absence of lytic principle by a simple method which will be described in another publication.

It will be noted in Table 1 that in three experiments the three and six drop quantities of lytic principle were effectively destroyed; that in two experiments under the conditions of the experiment, ten drop quantities of the bacteriophage were destroyed. Exposure of *B. coli* "D" to the ultra violet light under conditions of this experiment, results in no apparent effect upon this organism. These conditions are quite the reverse with other physical agents, for example heating to 56.5 degrees C. At this temperature *B. coli* "D" is killed but there is no action upon the lytic principle.

Experiments were also made with regard to the action of ultra violet light, under precisely the same conditions we employed with bacteriophage upon herpes virus and Levatidi's so-called encephalitis virus. In former experiments, by repeated passage from one animal to another, we had obtained a herpes virus (originally isolated from a case of *Herpes genitalis* by LeFèvre de Arric) which, when injected into rabbits intracerebrally or into the spinal canal in .2 cc. amounts of a 1:100,000 dilution would produce Herpetic encephalitis and death of the animal always within five days and usually within three days. The Levatidi so-called encephalitis virus, after repeated passage through many animals, obtained a fixed virulence, when .2 cc. of a 1:10,000 dilution of brain emulsion was employed, of five or six days. In both instances the original brain emulsion employed consisted approximately of one gram of brain emulsified in 2 cc. of physiological salt solution.

In these experiments the original brain emulsions were not

[†] We are indebted to Dr. A. F. Hess for the use of his Alpine Sun Lamp.

diluted. The concentrated emulsions were centrifuged at first to throw down any tissue particles which were present and the supernatants withdrawn for our experiments. Emulsions were made in both normal rabbit serum and in physiological salt solution. Two cc. of the supernatant containing each virus in serum and salt solution were placed in quartz tubes. These tubes were exposed to ultra violet light radiation in the same manner as were the tubes containing bacteriophage, for forty minutes at a distance of one foot, the tubes being agitated every eight minutes. After exposure of the viruses to ultra violet light, rabbits were injected subdurally with 0.2 cc. of the material from each tube. Control animals were injected with the untreated supernatants. (Table 2.)

TABLE 2.
Experiment 1.

Date	Rabbit No.	Brain emulsion tested	Amt. injected	Time exposed to ultra violet light	Date of Death	No. of days	Date of Living	No. of days	Remarks
1/27/26	2549	H2548 Serum	0.2cc.	minutes	2/2/26	6	2/26/26	30	
"	2550	H2548 Saline	"	40					
"	2551	E2547 Serum	"	40	2/1/26	5			
"	2552	E2547 Saline	"	40	2/18/26	22			
"	2553	H2548 Saline	"	0	1/31/26	3			Control
"	2554	H2548 Serum	"	0	1/31/26	3			"
"	2555	E2547 Saline	"	0	2/1/26	5			"
"	2556	E2547 Serum	"	0	2/2/26	6			"

Experiment 2.

2/6/26	2565	H2554 Saline	"	0	2/11/26	5	2/26/26	20	Control
"	2566	"	"	20			"	20	
"	2567	"	"	20			"	20	
"	2568	"	"	40			"	20	
"	2569	"	"	40			"	20	

H = Herpes virus.
E = Levaditi encephalitis virus

It will be noted that the control animals died within the specified time in experiments one and two; that the animals which received herpes and so-called encephalitis virus emulsified in serum and exposed to ultra violet light died in six and five days respectively; that the animal which received herpes virus emulsified in saline and exposed to ultra violet light was living and apparently normal thirty days after injection, while the rabbit which received the Levaditi virus which had been emulsified in salt solution and exposed to the ultra violet light, died twenty-two days after injection, as contrasted with five days for the control.

In Experiment 2 only the herpes virus was exposed to the action of ultra violet light and the emulsions were in saline. The control animal in this case died on the fifth day, while the animals receiving herpes virus which had been exposed to ultra violet light for twenty and forty minutes (the other conditions remaining the same as in the previous experiment) were living and apparently normal on the 20th day following injection.

While the series of animals in these two experiments is small, it is suggested that ultra violet light under the conditions we have worked is capable at least of attenuating both herpes virus and the Levaditi virus, and possibly destroys them completely under certain conditions. Further experiments are now in progress bearing on this problem. Since both herpes and Levaditi virus in serum brain emulsions are apparently protected from the ultra violet light, we thought it of interest to test bacteriophage under the same conditions. Experiments show that bacteriophage is also protected when serum is substituted for broth. The three, six and ten drop dilutions all remain active after exposure to the ultra violet light. Increase in density of the substrate may explain this.

Conclusions. One may say that under the conditions of these experiments, a lytic principle active for *B. coli* "D" is acted upon by ultra violet light in much the same way as are two strains of known filterable viruses, *i. e.*, herpes and Levaditi's so-called encephalitis virus. Exposure to ultra violet light at a distance of one foot for forty minutes is sufficient to attenuate or destroy both the bacteriophage and the two filterable viruses employed in these experiments. In a substrate of normal rabbit serum all three are protected from the action of ultra violet light.

2994

Further experiments upon the purification of pancreatic amylase.

H. C. SHERMAN, M. L. CALDWELL and MILDRED ADAMS.

[From the Department of Chemistry, Columbia University,
New York.]

In recent years much light has been thrown upon the chemical nature of some typical enzymes, both by the direct method of purification and analysis and by other methods which, while indirect, have proven none the less instructive.

Pancreatic amylase has been purified by the methods developed in this laboratory¹ to a maximum enzymic activity practically constant in many independent purification experiments. This material in the dry state is an amorphous white powder having the ultimate composition, and showing the color reactions of typical proteins. Moreover, the enzyme material showed, on analysis by the Van Slyke method,² the same forms of nitrogen as are found in typical proteins, and in typical quantitative proportions. Like the purified malt amylase previously described by Osborne,³ this pancreatic amylase on heating in water yielded a coagulated albumin and left in solution a proteose or peptone.

Our purified pancreatic amylase preparation has much the highest enzymic activity of any material of which we have found adequate record. In thirty minutes at 40°, this material splits 20,000 times its weight of starch and forms 10,000 times its weight of maltose; and notwithstanding its gradual inactivation in solution, this material has, in sufficiently long experiments, digested as much as 4,000,000 times its weight of starch and formed as much as 2,800,000 times its weight of maltose.⁴

This great enzymic activity was shown at a dilution of 1:100,000,000, whereas the most delicate tests for protein are probably not valid at dilutions greater than about 1:100,000. Thus the failure of protein reactions in solutions enzymically

¹ Sherman, H. C., and Schlesinger, M. D., *J. Am. Chem. Soc.*, 1911, **xxxiii**, 1195-1204; 1912, **xxxiv**, 1104-1111.

² Sherman, H. C., and Gettler, A. O., *J. Am. Chem. Soc.*, 1913, **xxxv**, 1790-1794.

³ Osborne, T. B., *J. Am. Chem. Soc.*, 1895, **xvii**, 587-603; 1896, **xviii**, 536-542.

⁴ Sherman, H. C., *Proc. Nat. Acad. Sc.*, 1923, **ix**, 81-86.

active does not show, as many writers assume, that the enzyme is of other than protein nature, since as in this case, the enzymic activity may constitute a test 1000-fold more delicate than any reaction which can be employed as a test for the presence of protein material.

The investigations of this laboratory upon pancreatic amylase have shown that this enzyme loses its activity in aqueous solutions, that this loss proceeds more rapidly with increase of temperature, and that it is undoubtedly connected with hydrolytic changes taking place in protein material which constitutes either the enzyme molecule itself or an essential part of it. Extensive studies of the conditions influencing the rate of hydrolytic inactivation of the enzyme, especially as affected by added amino acids, all give added evidence of the protein nature of the enzyme.^{5, 6}

This is further confirmed by the finding that the antiseptics which act chemically upon proteins are enormously more destructive of the enzyme than are the antiseptics of the lipoid-dissolving type.⁷

The amylase of malt (while more stable than that of the pancreas) has yielded qualitatively similar results; and it has also been possible in working with this more stable enzyme to show that it has a definite isoelectric point or zone and that the material which shows the enzymic activity is an ampholyte, presumably protein.⁸

Thus four different kinds of experimental evidence all point strongly and consistently to the protein nature of the enzyme.

Willstätter and his co-workers⁹ have questioned this conclusion. Largely by the use of methods involving adsorption with alumina gel and with kaolin, they have effected what they believe to be an unprecedented degree of purification of pancreatic amylase and have concluded that the enzyme as thus purified is of other than protein nature.

⁵ Sherman, H. C., and Walker, Florence, *J. Am. Chem. Soc.*, 1921, xliii, 2461-2469; 1923, xlv, 1960-1964.

⁶ Sherman, H. C., Caldwell, M. L., and Naylor, N. M., *J. Am. Chem. Soc.*, 1925, xlvii, 1702-1709.

⁷ Sherman, H. C., and Wayman, M., *J. Am. Chem. Soc.*, 1921, xliii, 2454-2461.

⁸ Sherman, H. C., Thomas, A. W., and Caldwell, M. L., *J. Am. Chem. Soc.*, 1924, xlvii, 1711-1717.

⁹ Willstätter, R., Waldschmidt-Leitz, E., and Hess, A. R. F., *Z. physiol. Chem.*, 1923, cxxvi, 143-169.

Valuable as is their work upon some aspects of the adsorption phenomena, their conclusions regarding the chemical nature of the enzyme and its quantitative activity as purified by their method are shown by our experiments to be in error. The outstanding source of error in their work is that, having reverted to the use of glycerol for the preservation of the enzyme in solution (which Osborne, and later we, had set aside in favor of procedures capable of yielding a dry product) they determined the activity of their glycerol-water solution of purified amylase and then interposed a long period of dialysis against running water before determining the (residual) solids for the calculation of the enzymic activity and testing them for the chemical nature of the enzyme. As we understand their account of their work, the tests upon which they base their conclusion regarding the nature of the enzyme were made upon material which exhibited no amylase activity. Their assumption that the substance of the enzyme (although inactivated) might be expected to remain in the dialyzer without material qualitative or quantitative alteration, we have found to be invalid.

In the work of this laboratory it has been repeatedly shown that pancreatic amylase in aqueous solution undergoes a relatively rapid hydrolysis into substances which are readily dialyzable and which therefore must have been lost by Willstätter, Waldschmidt-Leitz and Hess⁹ when they dialyzed for a week against running water, after making the enzymic test in their solution and before applying tests for the chemical nature of the enzyme, or attempting to ascertain its weight.

Hence it must be concluded that they greatly over-estimated the activity of their purified enzyme material because of greatly underestimating the amount of it present in the glycerol-water solution which they tested, and that their negative findings as to the chemical nature of the enzyme are inconclusive because of the long dialysis which they interposed between the tests for the activity of the enzyme and for its chemical nature had involved such serious losses that their subsequent tests cannot be regarded as having the significance which they attached to them.

We have carefully repeated their method of purification with painstaking precautions to establish and maintain the most favorable conditions for each step. The actual concentration of active enzyme relative to total nitrogen effected by their method in our hands is apparently about one-half that attained pre-

viously in this laboratory by the method of Sherman and Schlesinger. We have confirmed and extended many of their observations regarding the adsorption and elution of the enzyme, and, by careful determination of optimal conditions have obtained data which indicate that pancreatic amylase like malt amylase⁸ has a definite isoelectric point or zone at essentially the same pH at which it exerts its optimum enzymic activity.

These new experiments, including the direct repetition of the work of Willstätter and his co-workers, confirm the conclusion, reached in our previous investigations in which the problem of the chemical nature of pancreatic amylase has been approached from several different angles, that the enzyme either is of protein nature or contains protein as an essential constituent.

2995

Further proof of the "bios" character of crystalline bios 223.*

RALPH W. KERR, WALTER H. EDDY and R. R. WILLIAMS.

[*From the Laboratory of Physiological Chemistry, Teachers College, Columbia University, New York City.*]

In our original report¹ on the isolation and properties of Bios 223 we reported failure to obtain either the ninhydrin or mustard oil reaction for a primary amine, but did obtain a reaction with benzene-sulfon-chloride. In pursuit of the chemical structure of the crystals we have since obtained a reaction with HNO_2 and a Van Slyke nitrogen determination that checks our previous value obtained by the Dumas method. These results have not only given us new leads in the study of structure, but also a means of determining definitely whether the yeast growth stimulatory power resides in the crystals of Bios 223, or is due to contamination of the crystals by the active factor. The experimental data by which we have demonstrated that the crystals are responsible for the activity follow and constitute the subject of this report.

* To avoid confusion we have elected to identify our Crystalline Bios by using the melting point 223°C. in the designation.

¹ Eddy, Kerr, and Williams, R. R., *J. Am. Chem. Soc.*, 1924, xlv, 2846.

EXPERIMENTAL.

First Step: Preparation of an addition compound of Bios 223 and benzene-sulfon-chloride.

Two preparations of the sulfonamide have been made, identical in properties, one from a sample of Bios 223, isolated in September, 1925, and another from a sample isolated January, 1926. As shown by the sample exhibited, the compound is in the form of fine, white, needle shaped crystals melting sharply at 138.5° C. They were prepared as follows:

0.399 grams (0.003 moles) of a sample of Bios 223 were dissolved in the minimum amount of water. To this solution was added 0.48 grams of NaOH (4×0.003 moles) in 12 per cent solution. (N. B. Stronger solution of NaOH inhibited the formation of the compound.) To this mixture was then added, a little at a time, a total of 0.797 grams of benzene-sulphon-chloride. As this substance does not mix with water the combination was agitated from time to time over a period of 48 hours. At the end of this period the mixture was warmed to expel all traces of free b-s-c. The solution was then acidified with HCl and a precipitate of crude sulfonamide formed. Purification was accomplished by the following steps: The product being soluble in ether and relatively insoluble in acidified water its removal from the HCl solution was accomplished by shaking with ether, the sulfonamide passing into solution in the ether layer. This layer was removed and evaporated. The residue was next treated to eliminate any di-sulfonamides that might have been formed due to excess of b-s-c used. 8 cc. of sodium alcoholate solution (0.8 grams Na in 20 cc. of C_2H_5OH) was added directly to the residue and warmed for twenty minutes. Water was then added and the solution boiled to remove the alcohol. This left the product in solution as the sodium salt. Acidifying the water solution of the salt precipitated the compound again and by ether extraction it was freed from the water and the sodium chloride. The product was then purified further by crystallization from ether in which Bios 223 is insoluble, and two successive recrystallizations from hot water in which it is freely soluble. The yield was .220 grams of fine white needles melting sharply at 138.5° C. Successive recrystallizations failed to change the melting point. No known benzene-sulfonamide was found answering to this description. The compound is now under analytical investigation and data on the structure will be reported later.

Second Step: Tests for yeast growth stimulating power.

The product was then tested for activity. Clark's sugar-salt medium was used, the sugar being purified by recrystallization from 80 per cent alcohol. In each test tube was placed 8 cc. of medium, 1 cc. of water carrying the stimulatory material in definite dilution, and 1 cc. of a yeast suspension containing a definite and constant number of yeast cells (250,000) as determined by the hemocytometer. The tubes were incubated at 31° C. for 48 hours, the contents then transferred to a Hopkins vaccine tube and centrifuged at definite speed. The readings are given in fractions of cubic centimeters and in numbers of cells. *Neither the product obtained from the September, 1925, Bios 223 nor the January sample showed activity.* The replacement of a single hydrogen of the NH_2 group in Bios 223 by benzene-sulfon-chloride is sufficient to inactivate it. (See Table 1.) (N. B. To

TABLE I.
Sample activity tests on the benzene-sulfonamide of Bios 223.

Dosage	Results with September product		Results with January product.	
	Vols.	Cells.*	Vols.	Cells.
Controls	0.003 cc.	42	0.002 cc.	28
0.2 mg. sulfonamide per cc.	—	—	0.002	28
0.1 mg. sulfonamide per cc.	0.004	56	—	—
0.02 mg. sulfonamide per cc.	—	—	0.003	42
0.01 mg. sulfonamide per cc.	0.004	56	—	—
0.001 mg. sulfonamide per cc.	0.003	42	—	—

*A seeding of 250,000 cells per 10 cc. of combined culture medium, yeast suspension and test substance is called a count of 1. 42 cells then means that at the end of 48 hours' incubation the tubes contained $42 \times 250,000$ cells.

insure the presence of the benzene-sulfonamide in solution, the test fraction was first dissolved in hot water and on cooling did not precipitate out in the dilution used.)

Third Step: Restoration of activity by hydrolytic removal of the attached B-S-C Group.

Having now a crystalline compound of Bios 223 with no activity we proceeded to attempt its hydrolysis with sulfuric acid. Boiling the compound in 10 per cent sulfuric acid under a reflux condenser for six hours failed to split the compound. The hydrolysis was finally accomplished as follows:

100 mg. of the recrystallized, inactive sulfonamide was placed

in a tube with 8 cc. of water and 2 cc. of concentrated sulfuric acid and the tube sealed. The tube with its contents was then heated for one hour at 150° C. On cooling no precipitate formed and since the sulfonamide is difficultly soluble in cold acid solution, hydrolysis was assumed to have occurred. The hydrolysate was neutralized with baryta, filtered and evaporated to dryness. The residue contained considerable barium-benzene sulfonate. (Before testing this residue for activity the hydrolysate was slightly acidified with sulfuric acid and filtered. This split the barium from the sulfonate and removed all soluble barium salts.)

The hydrolysate residue was a mixture of benzene-sulfonic acid and bios but using this mixture definite evidence of activity was obtained. (See Table II.)

TABLE II.

Sample activity tests with the Bios 223, the sulfonamide made from it, and the hydrolysate residue left after splitting the benzene sulfonic acid from the inactive sulfonamide.

Dosage	Results A.		Results B.	
	Vols.	Cells.	Vols.	Cells.
Controls	0.003	42	0.003	42
0.1 mg. Bios 223 per cc.	0.016	227	0.017	245
0.01 mg. Bios 223 per cc.	0.004	56	0.005	71
0.2 mg. sulfonamide per cc.	—	—	0.002	28
0.02 mg. sulfonamide per cc.	—	—	0.003	42
0.2 mg. hydrolysate per cc.	0.009	128	0.011	156
0.02 mg. hydrolysate per cc.	0.004	56	0.004	56

Incubation 48 hours at 31 C.

CONCLUSIONS.

The isolation of a crystalline substance from an active substrate always leaves existent the doubt as to whether the activity is due to the crystals themselves or to adhering traces of the potent factor. The complete inactivation of Bios 223 crystals by forming the crystalline benzene sulfonamide and the recovery of activity by hydrolysing this inactive crystalline product seems to remove all reasonable doubt and to demonstrate that the yeast stimulating power of Bios 223 resides in the crystals themselves. The nature of the addition compound also suggested that the NH_2 group is concerned in the exercise of this function.

We also take pleasure in acknowledging the suggestions and advice of Professor John M. Nelson of Columbia University in developing these results.

2996

The pathway of nucleated erythrocytes introduced into the
splenic artery.

WARD J. MAC NEAL and MARJORIE B. PATTERSON.

[*From the Department of the Laboratories, New York Post-Graduate Medical School and Hospital, New York City.*]

Erythrocytes of the domestic fowl, washed free from plasma, were suspended in salt solution, one part in twenty. This suspension was then injected through a fine glass cannula into one of the short gastric branches of the splenic artery of a rabbit, under ether anesthesia. One minute after beginning the injection a portion of spleen at one end was clamped off, excised and placed in Helly's fixing solution for histological study. After two minutes a second sample was taken. After fifteen minutes the remainder of the spleen was perfused through the original cannula, first with a little salt solution and then with Helly's fixing solution, until the spleen became firm and yellowish in color.

Histological study of the one-minute sample showed abundant avian erythrocytes in the spleen pulp of the marginal zone of the Malpighian corpuscles with relatively less cells of this type in the spleen sinuses. At two minutes the relationship was similar but the number of nucleated erythrocytes in the sinuses and in the pulp at a distance from the Malpighian corpuscles had increased. In the last specimen, perfused with fixing solution at the end of fifteen minutes, the distension and perfect fixation permit a clear view of the intimate structural relationships. Here one sees the foreign blood cells in the pulp of the marginal zone and of the pulp cords but also in the interior of the sinuses. Some of them have already been ingested by phagocytic cells.

These observations are directly opposed to the view of Helly,¹ that all transfused foreign erythrocytes reach the spleen pulp after traversing the venous sinuses, and are quite in accord with the observations of Gray,² Mall,³ and Weidenreich,⁴ who have ascribed the conspicuous injection of the marginal zone of the

¹ Helly, Konrad, *Arch. f. mikr. Anat.*, 1902, lxi, 245.

² Gray, Henry, *Structure and use of the spleen*. London, 1854.

³ Mall, Franklin P., *Z. f. Morph. u. Anthrop.*, 1900, ii, 1.

⁴ Weidenreich, Franz, *Arch. f. mikr. Anat.*, 1901, lviii, 247.

Malpighian corpuscles to the termination of abundant arterial capillaries in the intercellular spaces of the splenic pulp in this region. These spaces evidently constitute a connecting link between the arterial and venous channels, significant for spleen function.

2997

The effect of "complete circulatory block" on the concentration of venous blood.

EDWARD TOLSTOI and ANNA J. EISENMAN.

[*From the Department of Internal Medicine of Yale University and the Medical Service of the New Haven Hospital, New Haven, Conn.*]

It is now well established that the production of venous stasis in a limb causes certain consistent alterations in the venous blood of the part, the most conspicuous of which is a loss of water, with a resulting hemo-concentration. Such a condition may also be produced by nearly completely cutting off the circulation in a limb for a period of 25 to 75 minutes. The latter fact was demonstrated by Dautrebande, Davies and Meakins¹ in 1923. However, since most of the reports on venous stasis were the results of short periods of compression, it was desirable to learn what change in the blood, if any, could be produced by nearly completely cutting off the circulation of a part for a short period. In other words, does nearly complete occlusion of the circulation of a limb for two or more minutes cause the blood of that part to concentrate? The results of our experiments answer that question and the data are given below.

Procedure and Methods. No breakfast was given to any subject. In all experiments the systolic pressure was first determined by means of a sphygmomanometer and the pneumatic cuff was inflated to keep the pressure 15 to 20 mm. above the systolic level. The duration of such stasis varied from 2 to 5 minutes. In two subjects the compression was relieved at the end of two minutes; in one at the end of three; and in two others at the end of

¹ Dautrebande, W., Davies, H. W., and Meakins, J., *Heart*, 1923, x, 133.

five minutes. No subject complained of actual pain. All, however, described a tingling sensation or that of "pins and needles" in the limb about which the cuff of the sphygmomanometer was wrapped. After maintaining the pressure at a level sufficient to produce nearly complete circulatory occlusion for the desired period of time, blood was drawn from a vein at the bend of the elbow. A 20 cc. luer syringe was used into which some sterile mineral oil was placed to prevent the blood from coming into contact with air. At times it was difficult to obtain the 20 cc. of blood desirable for analytical purposes. After the first 10 cc. were withdrawn, the blood flowed more slowly and on two occasions it was necessary to withdraw the needle from the vein and proceed with the material available, as no more could be obtained from that particular vein. The cuff was released immediately before the withdrawal of the needle. The blood thus obtained was then placed over mercury in a blood sampling tube. About 3 cc. of mercury were left in a 20 or 25 cc. bulb, and this was used to defibrinate the blood, by repeatedly inverting and keeping the sampling tube in motion for about 10 to 15 minutes. For the controls, blood was drawn from the opposite arm, and treated in a similar manner. Both bloods were then studied for their respective oxygen capacity, cell volume, and serum proteins, employing the same technical methods described in a former paper.²

The results of our experiments are presented in the accompanying table. Their striking feature is the lack of any detect-

TABLE 1.

Experi- ment No.	Cell volume	Oxygen capacity	Serum proteins	Remarks.
	<i>vols. per cent</i>	<i>vols. per cent</i>	<i>per cent</i>	
1	42.5	17.95	6.94	Constriction for 2 minutes
	42.8	17.95	6.93	Control
2	50.0	21.0	7.57	Constriction for 2 minutes
	50.1	21.2	7.56	Control
3	42.0	17.2	7.53	Constriction for 3 minutes
	42.2	17.3	7.52	Control
4	47.0	20.5	7.42	Constriction for 5 minutes
	47.0	20.4	7.42	Control
5	48.0	21.80	7.67	Constriction for 5 minutes
	47.5	21.85	7.70	Control

² Peters, J. P., Eisenman, A. J., and Bulger, H. A., *J. Clin. Invest.*, 1925, **v**, 435.

able change in the constituents studied. No change whatever was observed in bloods taken from parts in which the circulation was cut off as compared with bloods taken from the opposite limb without any stasis.

A satisfactory explanation of this observation is difficult, since longer periods of similar type of occlusion cause demonstrable alterations in the water content of the blood. Venous stasis for short periods also causes like changes, due to a transfer of water from the blood to the tissues. If the underlying process causing the fluid shift is a mechanical one, namely filtration, then the explanation of our results on that basis may prove possible. The establishment of venous stasis causes after a short period, an increase of tension in the obstructed vein, such an increase being due to the constant inflow of blood from the artery. As the duration of the stasis is increased the pressure within the vein rises and probably causes a filtration of water, and a resulting hemo-concentration. When, however, both the artery and vein are obstructed for *short* periods as we have done, then no changes are detectable, because no great alterations of the intravenous tension are possible in so short a period, since little blood can flow from the constricted artery. Should the above explanation be tenable how then can we explain the results of Dautrebande, Davies and Meakins, that is, cutting off the circulation for long periods of time and obtaining a concentration of the venous blood?

In a recent publication Lewis and Grant³ claim that it is not always possible to completely occlude the circulation with a 12 cm. armlet such as is ordinarily used for blood pressure determinations. No matter how snugly such a pressure cuff is applied and how greatly inflated, such a procedure will not "always exclude a very slow trickle of blood through bone anastomoses into the lower part of the arm." Since the return of blood is through the veins and since the largest veins of the arm are easily compressible so that venous return is blocked, it is not difficult to appreciate the fact that during a long period of so called "complete occlusion" blood slowly flows into the veins, increases the tension therein with the resulting filtration of water and a concentration of the venous blood. Such a hypothesis may explain why a long period of so called "complete occlusion" will

³ Lewis, T., and Grant, B., *Heart*, 1925, xii, 1, 73.

produce changes in the venous blood similar to those caused by short periods of venous stasis, while short periods of "complete" block (2 to 5 minutes) show no change in blood concentration.

This explanation assumes that hydrostatic pressure is the only factor that produces exudation of fluid from the blood. This is obviously not the case. As Dr. J. P. Peters² pointed out to us, osmotic and acid-base changes must be important factors. The production of tissue acidosis as a result of asphyxia should lead to a transfer of water from the blood to the tissues. It may be that this asphyxia does not become serious enough in short periods to exhibit its effect in the blood. In this connection it must be appreciated that the circulation in these experiments was at a standstill and changes in the tissues could affect the blood of the anti-cubital veins only by a process of diffusion through a considerable distance.

The practical application of our observation should be appreciated. The concentration changes which occur when blood is drawn by the usual method from a compressed vessel have been pointed out. We merely wish to emphasize the desirability of drawing blood either *without* stasis or with "complete occlusion where concentration changes are to be carried out; especially where determinations of such constituents as serum proteins, chlorides, and blood gases are to be determined.

SUMMARY AND CONCLUSIONS.

Studies were carried out on venous bloods obtained from limbs in which the circulation was cut off for periods of 2 to 5 minutes by means of a sphygmomanometer cuff. Such bloods were studied for their cell volume, oxygen capacity, and plasma proteins. Control bloods were obtained from the opposite limb where no compression was made.

No changes in the constituents studied were observed in cases where "complete compression" was used and we are forced to conclude that short periods of so called "complete block" do not produce a shift of water either from or to the tissues.

The practical application of the above is emphasized.

2998

**Blood sugar in status thymo lymphaticus: new theory as to
cause of sudden death.**

AUBREY B. MacLEAN and RUTH C. SULLIVAN. (Introduced by
M. Wollstein),

*[From the Chemical Laboratory of the Babies' Hospital and the
Department of Diseases of Children, College of Physicians
and Surgeons, Columbia University, New York City.]*

In the majority of patients who died with symptoms of status thymo-lymphaticus in the wards of the Babies' Hospital, the prostration of the patient was so marked that it was out of all proportion to the general physical findings. The clinical picture closely simulated a state of sugar shock from overdosage of insulin. It was, therefore, decided to determine the blood sugar level in such patients. In three cases where the diagnosis of status thymo-lymphaticus was confirmed by autopsy a sample of blood was taken within half an hour of death while the patient was in convulsions. The blood sugars were 52, 57, and 42 mg. per 100 cc. (Table I.) All three are considerably below the normal blood sugar of infants, which we have found to be between 80-120 mg. per 100 cc. as estimated by the Myers-Bailey modification of the Lewis Benedict method.

We have determined the blood sugar within half an hour of death in a series of patients with diseases other than status thymo-lymphaticus. (Table II.) The values are all normal or above normal. Hypoglycemia is not therefore a constant finding before death.

The blood sugars of six patients in convulsions of some hours' duration produced by conditions other than status thymo-lymphaticus were normal or above normal. (Table III.) It does not therefore seem probable that the convulsions alone produced the hypoglycemia.

The blood sugar was estimated in six cases of suspected status thymo-lymphaticus where roentgenograms showed a large thymus. The patients were not in convulsions at the time the sample of blood was taken. With the exception of one, which was slightly decreased, the blood sugars were normal. (Table IV.)

TABLE I.
Blood sugar of thymo-lymphaticus patients during convulsions immediately preceding death.

Case	Age	Sex	Time in convulsion before taking blood	Blood chemistry in mg. per 100 cc.				Spinal fluid in mg. per 100 cc.		Cistern fluid in mg. per 100 cc.	
				Sugar	Chlorids	Urea N	CO ₂ OP	Sugar	Chlorids	Sugar	Chlorids
I	VOB 24 mo.	F	5½ hours	52	—	20.8	27 vols per cent	28	650	—	—
II	ME 16 mo.	M	2 hours	42	550	—	—	62	738	64	725
III	TL 22 mo.	M	drowsy 12 hours	57	600	—	—	26	775	—	—

TABLE II.
Blood sugars done within half an hour of death in patients suffering from diseases other than status-thymo-lymphaticus.

Case	Age	Sex	Diagnosis	Blood sugar mg. per 100 cc.	Spinal fluid sugar mg. per 100 cc.	Remarks.
I	2 yrs.	M.	Anemia Lead poisoning	188	94	Autopsy performed by medical examiner. Only cause of death anemia. Suprarenal normal. Spleen negative. Thymus 15 gm. Lymph nodes normal. Convulsions for 3 hrs. before death. Twitching of muscles of extremities. In state of coma.
II	5½ yrs.	F.	Acute intestinal intoxication	202	—	
III	8 mo.	M.	Tuberculosis of cerebral and spinal meninges	141	12	
IV	10 mo.	M.	Acute intestinal intoxication	101	—	
V	9 mo.	F.	Mongolian idiocy Acute nephritis Bronchopneumonia	93	—	
VI	3 yrs.	F.	Acute catarrhal colitis Tuberculosis of cerebral and spinal meninges	165	28	

TABLE III.
Blood sugars of patients in convulsions produced by other conditions than status-thymo-lymphaticus.

Case	Age	Diagnosis	Time in convulsions before taking blood	Blood chemistry in mg. per 100 cc.				Sugar	Chlor.	Result	Necropsy
				Sugar	Chlor.	Ca.	P.				
I	8 mos.	Bronchopneumonia	8 hrs.	140	650	10.5	3.5	130	675	Died	Thymus 5 gm. Spleen neg. Suprarenal normal.
II III	6 mos. 12 mos.	Tetany Tetany	6 hrs. 24 hrs.	106 130	500	7.7	4.0	86	673	Improved Died	Thymus 5 gm. Spleen neg. Suprarenal normal.
IV V	5 mos. 5 yrs.	Tetany Streptococcus Meningitis	4 da. 2-3 hr. 3 hrs.	106 134		5.2	5.3	71 19	763 500	Not impr. Died	Autopsy performed by the medical examiner who gave the only cause of death as: Anemia. Suprarenal normal. Spleen neg. Thymus 15 gm. Lymph nodes normal.
VI	2 yrs.	Anemia Lead poisoning	3 hrs.	188	538			94	675	Died	

TABLE IV.
Blood sugar of patients having large thymus as shown by roentgenogram at a time when they were not in convulsions.

Case	Age	Sex	Diagnosis	Blood sugar in mg. per 100 cc.	Remarks
I	21 mos.	F	Hypertrophied Tonsils Hyperplasia of Thymus	106	No history of convulsions
II	3½ mos.	F	Enlarged Adenoids Hyperplasia of Thymus	72	History of convulsions
III	4 mos.	M	Status Lymphaticus Rickets	111	No history of convulsions
IV	6½ mos.	M	Hyperplasia of Thymus Acute Intest. Intoxication	86	No history of convulsions
V	13 mos.	M	Hyperplasia of Thymus Acute Tonsillitis	89	No history of convulsions
VI	4 mos.	M	Enlarged Thymus	103	Difficulty in breathing

The hypoglycemia, therefore, was temporary rather than continuous.

Acute suprarenal insufficiency is suggested as the immediate cause of the sudden death in status thymo-lymphaticus.

2999

Comparative study of action of antiseptics on staphylococci and body cells by tissue culture method.

R. A. LAMBERT and JUVENAL R. MEYER.

[*From the Department of Pathological Anatomy of the Faculty of Medicine, Sao Paulo, Brazil.*]

Experiments begun in 1916 by one of the authors¹ to test *in vitro* by the tissue culture method the comparative action of certain bacterial substances on tissues and on bacteria have been continued. In the previous studies human tissues were used and a uniform exposure of one hour was employed. In the present experiments rabbit tissue (spleen) was used throughout, and the time of exposure in the first series of experiments was twenty minutes. The antiseptics tested have included alcohol, iodine, mercuric chloride, mercurochrome, acriflavine, protargol, albargin, gentian violet, neosalvarsan, and hexylresorcinol.

The spleen of a freshly killed rabbit was divided into pieces of about 1 mm. in diameter. After washing in physiological salt solution, one lot of the fragments was placed one minute in a suspension of *Staphylococcus aureus*, and then put in graded solutions of the antiseptic for 20 minutes. Tissue cultures were then prepared in hanging drops of homologous plasma, following two washings of the tissue in salt solution. A second set of cultures were made from non-infected tissues similarly exposed with appropriate controls of untreated tissues. The comparative effect of these antiseptics on splenic cells and staphylococci is shown in the following table.

It is seen that in the majority of instances, particularly in the case of several of the newer antiseptics, bacteria proved decidedly

¹ Lambert, R. A., *J. Exp. Med.*, 1916, xxiv, 683.

	Cells survive	Staph. survive	Cells killed by	Staph. killed by
Alcohol	20 per cent	33 per cent	33 per cent	50 per cent
Iodine	1:2500	1:2500	1:1000	1:1000
Mercuric chloride	1:10,000	1:20,000	1:2500	1:10,000
Mercurochrome	1:1-2000	1:500	1:500	1:250
Acriflavine	1:20-40,000	1:10-20,000	1:10,000	1:5000
Protargol	1:500	1:250	1:250	1:100
Albargin	1:1000	1:500	1:500	1:250
Gentian violet	1:250,000	1:25,000	1:100,000	1:10,000
Neosalvarsan	1:1000	1:2000	1:500	1:1000
Hexylresorcinol	1:5000	1:2000	1:2000	‡ *

* Owing to the slight solubility of hexylresorcinol in water (*i. e.*, physiological salt solution) it was not possible to complete these tests.

more resistant than cells. The most favorable results were obtained with iodine, mercuric chloride, and neosalvarsan. It is possible, however, that even in these cases the surviving tissue cells may have come from the center of the small fragments where they were partially protected.

A second series of experiments was carried out in which the antiseptic was added directly to the plasma culture medium. The preparations were divided into two lots, one of which was infected with staphylococci. As was expected, a toxic effect against both cells and bacteria was obtained with considerably higher dilutions than in the first series, exposed only twenty minutes. In the second series neosalvarsan was the only agent which appeared definitely less toxic for cells. Inhibition of bacterial growth was seen in dilutions of plasma of 1:30-40,000, while the cells were only slightly damaged by these dilutions. The findings with acriflavine and mercurochrome were quite as unfavorable as in the twenty-minute tests.

The two chief types of cells in the cultures—large mononuclears and fibroblasts—seem to be about equally resistant to the various agents. In a few treated preparations fibroblasts were seen without mononuclears, while in several the reverse was noted.

In general the tests suggest that in so far as the direct action on cells and staphylococci are concerned, some of the classic antiseptics, such as iodine and mercuric chloride, approach nearer the ideal than certain of the newer preparations, and that neosalvarsan might be hopefully employed in generalized bacterial infections as well as in certain protozoan diseases.

3000

The physiological behavior of glucosane.**H. J. DEUEL, JR., J. A. MANDEL, and S. S. WADDELL.**

[From the Department of Physiology, Cornell University Medical College, and the Department of Biochemistry, New York University and Bellevue Hospital Medical College, New York City.]

The anhydrosugar, glucosane ($C_6H_{10}O_5$), and its polymer, tetra-glucosane, are widely used in Germany in the dietary treatment of diabetes. These substances are reputed to be oxidized by the diabetic since, according to Nothmann and Kühnau¹ and others, no extra urinary sugar results after its administration, no rise in blood sugar occurs thereafter, and an antiketogenic and protein sparing action follows its ingestion. It is likewise claimed to be glycogenic.

In the present paper we have investigated the behavior of "Salabrose," a commercial tetra-glucosane preparation obtained from Germany, and have administered it orally, subcutaneously, and intraperitoneally to normal and phlorhizinized dogs. In order to determine whether glucosane might be excreted unchanged in the urine or feces following its administration, it has been quantitatively determined by reconversion to glucose by boiling for a short interval with dilute mineral acid. It was found that boiling for 15 minutes with 20 or 30 per cent HCl destroyed appreciable quantities of this substance, while boiling with a HCl concentration of 5 per cent of the same length of time completely hydrolyzed it to glucose. After neutralization the glucose obtained by this hydrolysis was determined by the usual methods. In diabetic urines this procedure was found to be satisfactory, the glucosane being equivalent to the total glucose (after hydrolysis) minus the preformed glucose.

When 30 gram doses of salabrose were fed to normal or phlorhizinized dogs no increase in the glucose excretion was noted, a result in harmony with the earlier reported German work. However, no rise in respiratory quotient was noted in normal or phlorhizinized dogs following its oral administration,

¹ Nothmann, M., and Kühnau, J., *Die Therapie der Gegenwart*, 1925, ix, —

indicating that only a slight oxidation, if any, occurred after its ingestion. The same amount of glucose, when fed to a fasting dog, resulted in a prompt rise in respiratory quotient.

On seeking an explanation of the fate of this substance it was found that amounts often larger than 50 per cent of that ingested were excreted unchanged in the feces. Often times diarrhea resulted following its ingestion, but even when this did not happen large amounts were found unchanged in the feces. Five to 10 per cent of the total amount ingested was usually found in the urine, during the first 6 hours after its ingestion, while no additional measurable amount was excreted in the later hours. The 30 or 35 per cent unaccounted for is probably excreted slowly in the urine or feces or may possibly in part be destroyed by intestinal bacteria.

In order to determine whether glucosane is capable of being utilized in case it were absorbed, it was injected intraperitoneally and subcutaneously in normal dogs. In both cases the glucosane was excreted unchanged in the urine, a complete recovery being obtained in from one to three days thereafter. These latter experiments confirm those previously discussed in indicating that glucosane can neither be changed to glucose nor oxidized as such in the dog. If glucosane be oxidized in diabetic patients it can be proved only by the demonstration of an increased respiratory quotient in the diabetic following its ingestion, coupled with the proof of its absorption from the alimentary tract and its non-elimination by the kidneys.

3001

The prevention and cure of tetany by oral administration of
magnesium lactate.

W. F. WENNER. (Introduced by W. W. Swingle).

[*From the Osborn Zoological Laboratory, Yale University,
New Haven, Conn.*]

It was demonstrated in a recent series of experiments¹ that strontium lactate administered orally to parathyroidectomized dogs greatly delays the onset of tetany, and may even bring

¹Swingle, W. W., and Wenner, W. F., *Am. J. Physiol.*, 1926, **lxxv**, 372-8.

about a permanent cure if the treatment is long continued. Preliminary experiments, carried out in conjunction with the strontium work, indicated that magnesium salts act similarly to strontium. The present paper is concerned with this phase of the work.

If parathyroidectomized dogs are given a 5 per cent solution of magnesium lactate in 100 cc. doses three times daily by stomach tube, they may be kept free from tetany for long periods, occasionally for three weeks. Some of the animals may develop tetany after eight or ten days. Once tetany becomes manifest, and the serum calcium falls below 7 mg. per 100 cc., the magnesium exerts little effect except to allay the more violent symptoms, probably through its anesthetic effect. However, if the animal is fed a pint or more of milk each day, along with the magnesium, the tetany soon disappears, generally within three or four days, and if the milk is discontinued the dog remains normal and shows no tetany symptoms so long as the magnesium is administered. Examination of the serum calcium shows that the calcium has risen to 7.5 or 8 mg. per 100 cc., which is above the level at which tetany becomes manifest in dogs.

The experiments indicate clearly that magnesium, *per se*, exerts little influence on tetany except through its action in sparing calcium. Somehow, the magnesium prevents the serum calcium from falling below the tetany level. A striking illustration of the sparing action of magnesium on calcium is shown in the following experiment. Parathyroidectomized dogs were allowed to develop tetany and then given the regular magnesium treatment. The animals died within a few days although the more violent symptoms of tetany were held in check. On the other hand, if tetany dogs are given several hundred cc. of calcium lactate by mouth, and this is followed by magnesium treatment, many of the animals do not again show tetany symptoms. The calcium raises the level of this salt in the blood and the magnesium somehow serves to maintain the level above 7 mg. per 100 cc. In the absence of magnesium, the calcium level of the blood soon falls and tetany appears.

It is a curious fact that if magnesium treated dogs are kept free from tetany for forty days, a large per cent of them permanently recover from tetany and may be placed on a heavy meat diet without ill effects. Thus ten dogs were used in this experiment; animals 2, 3, 7, 8 and 9 completely recovered and read-

justed themselves to loss of their parathyroids. Dog 6 remained free from tetany for ten days after operation but escaped from the laboratory before completion of the experiment. All of the animals developed tetany some time during the experiment, for if the tetany symptoms did not develop spontaneously by the third week, the magnesium was discontinued and the animals were fed meat until symptoms appeared.

3002

The menstrual cycle in the monkey; effect of double ovariectomy and injury to large follicles.*

EDGAR ALLEN.

[From the Department of Anatomy, University of Missouri School of Medicine, Columbia, Mo.]

In the course of some experimental work upon the effect of injections of ovarian and placental hormones into monkeys, operations have been performed at several intervals of the menstrual cycle. In four monkeys both ovaries were removed; in a fifth, large follicles were injured. The effect of these operations upon the time of appearance and the duration of the next menses provide some evidence as to the cause and nature of menstruation.

The ovaries were removed from the first monkey on the first day of the menstrual cycle (dated from the appearance of bleeding). The operation apparently had little effect for the menses continued to the fourth day. No second bleeding followed this one during a control period.

Double ovariectomy was performed upon the second and third monkeys on the 10th and 14th days of the cycle respectively. At this time the skin of the external genital organs and surrounding regions was considerably reddened. Ovulation had recently occurred in both cases and the corresponding tubes were removed and the ova recovered.¹ Apparently typical menstrual bleeding

* This work has been assisted by a grant from the Committee for Research on Sex Problems of the National Research Council.

¹ Allen, Edgar, *PROC. SOC. EXP. BIOL. AND MED.*, 1926, **xxiii**, 381.

began on the 4th day after operation in the second monkey and on the 5th day in the third monkey. The duration of these menses was 8 and 3 to 4 days respectively.

Both ovaries were removed from a fourth monkey at a time when the regions surrounding the genitalia were greatly swollen and reddened. Vaginal smears for several days before the operation were free from leucocytes. Although the time of operation in this animal cannot be dated in the cycle from an observed menstruation, the condition described would indicate a time near or after the middle of the cycle, probably included between the 10th and 20 days. Upon sectioning, the ovaries were found to contain several large follicles. Bleeding began on the 4th day after operation and lasted 7 days.

In the fifth monkey, operated on the 16th day of the cycle, one ovary appeared large and opalescent but no individual follicles could be distinguished from the surface. This ovary was pierced longitudinally by a sterile capillary pipette and some clear liquor aspirated. This was done without handling the ovary or interfering with the blood supply, for the ovary was held by forceps grasp of the ovarian ligament. The other ovary, which was small and opaque, was not disturbed. Bleeding began on the 6th day after the operation and lasted 6 days.

In the first four monkeys from which both ovaries had been removed the reddening and swelling of the vulva and surrounding regions had quite completely disappeared by the middle to the end of the second week following the operation.

Effect of double ovariectomy (1st four cases) and injury to large follicles (last case) on the next menses.

Monkey	Date of operation	Time of operation; day of cycle	Appearance of menses; day after operation	Duration of menses in days
(1) SLE	8/7/25	1st	Already bleeding when operated	4
(2) NS	11/21/25	10th	4th	8
(3) SRE	9/2/25	14th	5th	3-4
(4) S2E	7/21/25	10th-20th	4th	7
(5) SB	1/2/26	16th	6th	6

In performing these operations special care was taken to avoid injuring the uterus. The period elapsing between operation and hemorrhage (in all but the first case) and the duration of hem-

orrhage exclude operative technique as a contributing cause in the results described.

To summarize briefly: (1) Removal of both ovaries on the first day of menstruation had no apparent effect on that period. (2) Double ovariectomy or injury to large follicles dated toward the end of, or immediately after, the follicular phase of the cycle was followed by apparently typical menstrual bleeding. These experimental menses appeared from 5 to 13 days before the expectation as calculated by the length of previously observed cycles and by Corner's mode² for cycle length of 27 days. (3) External coloring and swelling disappeared after double ovariectomy.

From these data it is concluded that menstruation is probably due to an absence of follicular hormonal stimulus after it has been acting for a certain period of time. In this connection it should be noted that the presence of the follicular hormone has been demonstrated in human *corpora lutea*.³ It is quite possible that the corpus of the monkey may also continue to secrete the follicular hormone, thus postponing the onset of menstruation.

3003

Extirpation experiments upon the embryonic forelimb of the rat.

J. S. NICHOLAS.

[From the Department of Anatomy, School of Medicine, University of Pittsburgh, Pittsburgh, Pa.]

The technique of intrauterine surgery by which experimental methods may be applied to mammalian embryos has recently been described.¹ The results of a preliminary investigation show that rat embryos, operated within the last eight days of development, generally survive the operation, remain viable and are born with the rest of the litter. It is possible to rear such animals although it is difficult to do so.

² Corner, George W., *Carnegie Inst. Cont. to Emb.*, 1923, xv, 73.

³ Allen, Edgar, *Proc. Soc. Exp. Biol. and Med.*, 1925, xxii, 303.

¹ Bors, Ernst, *Roux's Arch. f. Entw.*, 1925, iii.

² Gerard, R. W., *Proc. Am. Soc. Physiol.*, 1926.

³ Nicholas, J. S., *Anat. Record*, 1925, xxxi, 4.

The first point of attack to which this method is being applied is the study of the factors influencing limb development. The first phase of this problem, the capacity for regeneration, forms the basis of this communication.

The anesthetized pregnant animal is placed upon the operating table with the dorsum toward the operator. An incision is made extending from the costal line to the anterior margin of the hind leg, through the dorsal skin and musculature of the body wall. This admits the operator to a field in which the embryos are clearly visible through the uterine wall. This method of approach obviates excessive handling of the viscera and consequent operative shock.

The incision through the uterine wall is made just large enough to permit manipulation of the limb and its extrusion through the opening. The limb is then excised at the determined level by means of fine iridectomy scissors, ligature or cautery.

Series I—Complete amputation of the limb at the scapulo-humeral junction. (See Table I.) The limb subsequent to the twelfth day of development shows all essential details. The manus is well formed, the digits even in the youngest stages are clearly outlined at the time of operation.

This series comprising 104 operated animals (which in every case were permitted to complete the gestation period subsequent to the operation) gave absolutely negative results in regard to the power of regeneration possessed by the embryo of this form during the final third of the gestation period. There is superficial healing and the wound is closed within a relatively short time after the operation. The processes involved in healing are purely superficial in nature in all cases examined, being limited to the layers of the body covering. The deeper tissues belonging to the limb and to its girdle show no excessive proliferation.

Series II—Amputation of the limb at the junction of the arm and forearm. Similar results were obtained to those in the preceding series. Healing is more complete. A pad of connective tissue is formed over the stump and a callus is formed at the extremity of the bone wounds. No other indications of regenerative capacity are found. These results are based upon the gross study of 32 cases preserved at birth and two cases which have undergone further development, one to 90 days of age. In this single individual there is nothing to add to the conditions

found in those taken at birth except that a bursa has developed in the connective tissue forming the pad of the stump.

Series III—Amputation of the manus at the radio-ulner articulation. A series of 18 cases, see table, shows the same healing power but the complete absence of regeneration of lost parts.

Series IV—Amputation of digits. The tissues removed at operation must be regarded as having the greatest prospective potency, from the standpoint of tissue age, of any of the series. The digits are among the last elements of the appendage to undergo complete development and should therefore retain their

Series I—Total amputation of arm.

Age of embryo in days.	Days before birth.	Number of cases.
14	7	14
15	6	16
16	5	7
17	4	22
18	3	16
19	2	11
20	1	18

Series II—Amputation of the limb at the junction of the arm and forearm.

Age of embryo in days.	Days before birth.	Number of cases.
15	6	6
16	5	3
18	3	9
19	2	10
20	1	6

Series III—Amputation of the manus at the radio-ulner junction.

Age of embryo in days.	Days before birth.	Number of cases.
14	7	8
16	5	4
18	3	3
19	1	3

Series IV—Amputation of digits.

Age of embryo in days.	Days before birth.	Number of cases.
14	7	4
15	6	2
17	4	1
20	1	1

embryonic potency longer than those tissues entering into arm or forearm development at any given stage.

In so far as the gross study of the animals of this series is concerned, they show no more than do the preceding series concerning the potentiality of regeneration. Tissue proliferation and the steps in the healing of wounds in the embryo are now being studied histologically. These studies will form a separate communication.

The present results show that in rat embryos, operated subsequent to the fourteenth day of gestation, there is not regeneration sufficient to restore an amputated limb. The embryonic age at the time of operation represents two-thirds of the gestation period in this form. If, therefore, there exists a period in the rat embryo during which it is capable of reforming or replacing an amputated limb this potency must be restricted to a stage earlier than those here recorded.

3004

Fixed base in gastric juice.

JAMES L. GAMBLE and MONROE A. McIVER (with assistance of Pauline Marsh and Elizabeth Matteson).

[*From the Department of Pediatrics and of Surgery, Harvard Medical School, Boston, Mass.*]

The authors in a study of the effects of pyloric obstruction in rabbits¹ noted a large loss of fixed base as well as of chloride ion into the stomach. Gamble and Ross² found sodium in vomited stomach secretions from a dog, following experimental obstruction of the pylorus, to the extent of about one-half the equivalence of the chloride ion loss. The experiments to be here reported were undertaken for the purpose of determining whether or not fixed base is contained to an appreciable extent in gastric juice produced under conditions permitting normal gastric function. So far as we are aware the only published measurements of

¹ Gamble, J. L., and McIver, M. A., *J. Clin. Invest.*, 1925, i, 531.

² Gamble, J. L., and Ross, S. G., *J. Clin. Invest.*, 1925, i, 403.

fixed base in gastric juice are those of Rosemann.³ These were obtained from two large samples collected from dogs by the "sham feeding" method of Pawlow. The average amount of fixed base found in this "hunger juice" was 20 cc. 0.1 N per 100 cc., and of this approximately 11 cc 0.1 N was sodium and 9 cc. 0.1 N potassium. The chloride ion content of the samples was in average 147 cc. 0.1 N. The secretion of an alkaline juice by the mucosa of the pyloric antrum was noted by early workers and has recently been confirmed by several investigators.^{4, 5} No measurements of fixed base in this secretion have been reported.

In this study gastric secretions were obtained from isolated pouches constructed in the fundic and pyloric regions of the stomach. These pouches were provided with external fistulae but were found to retain the secretions entering them until removed by catheterization. In the table are given data obtained from samples of juice withdrawn from a pouch in the fundus at intervals following ingestion of high protein food (meat or fish) and of low protein food (cereal and cream or bread and water). Juice taken from the pouch in the morning before food was given was also analyzed. As may be seen in the table the chief finding from these three groups of data is a wide range of fixed base concentration in the presence of a fairly constant value for chloride ion. The amounts of fixed base found in juice from the pouch during digestion of high protein food in the stomach are in average somewhat larger than Rosemann found in "hunger juice" from dogs. A much higher level of fixed base obtains following ingestion of low protein food. There is thus indicated the probability that, with a nearly constant chloride ion concentration, the fixed base level is adjusted in terms of the amount of ingested material capable of binding chloride ion. The "morning specimens" should not, we believe, be regarded as consisting entirely of secretions produced during fasting. It was found that when the pouch was emptied late in the preceding evening no juice could be withdrawn from it the following morning. If, however, it was drained during the afternoon, morning specimens of varying size were obtained. These probably consist of juice from an unfinished period of gastric function mixed with the

³ Rosemann, R., *Arch. ges. Physiol.*, 1907, cxviii, 467.

⁴ Ivy, A. C., and Ayama, Y., *Am. J. Physiol.*, 1921, lvii, 51.

⁵ Limb, R. K. S., and Dott, N. M., *Quarterly J. Exp. Physiol.*, 1923, xiii, 159.

mucous and faintly alkaline secretion of the resting fundus.^a This surmise is supported by the larger amounts of fixed base found in the smaller specimens, and also by the presence of a slight milkiness in contrast with the water-clear character of the specimens obtained during active digestion in the stomach. The measurements of potassium given in the table indicate a roughly stationary value for this base factor. The pH of the samples obtained during digestion of food was beyond the reading range of the indicator at hand, thymol blue, *i. e.*, was less than pH 1.2. The pH of one of the morning specimens, No. 3, was 2.2. The organic substance (dried substance—ash) was, for a combined specimen composed of several morning collections, 0.265 gm.

Food	Spec. No.	Interval after food hrs.	Amount of juice cc.	Cl'	B.	Cl'·B.	K.
				cc. 0.1 N	cc. 0.1 N	cc. 0.1 N	cc. 0.1 N
Meat	1	1	2	160	22	138	
	2	2	7	166	17	149	
	3	3	4	167	24	143	
	4	6	8	168	44	124	
Fish	5	2	9	156	31	125	12.5
	6	4	3	157	38	119	
	7	2	9	171	32	139	
	8	2	11	169	30	139	11.7
	9	2	21	173	24	149	11.8
	10	4	3	152	35	117	11.6
Averages				164	30	134	12
Cereal and Cream	1	6	10	160	72	88	14.6
	2	6	14	162	77	85	10.4
	3	8	8	157	83	74	9.6
	4	6	7	151	80	71	8.9
	5	2	4	139	34	105	
	6	2	8	167	71	96	10.9
Bread & Water	7	2	4	165	65	100	
	8	2	6	162	48	114	
Averages				158	66	92	11
Morning Specimens	1		24	155	72	83	12.1
	2		13	158	89	69	
	3		5	164	142	22	11.8
	4		8	157	138	18	15.6
Averages				157	110	47	13

Data from samples of gastric juice taken from an isolated pouch in the gastric fundus of a cat.

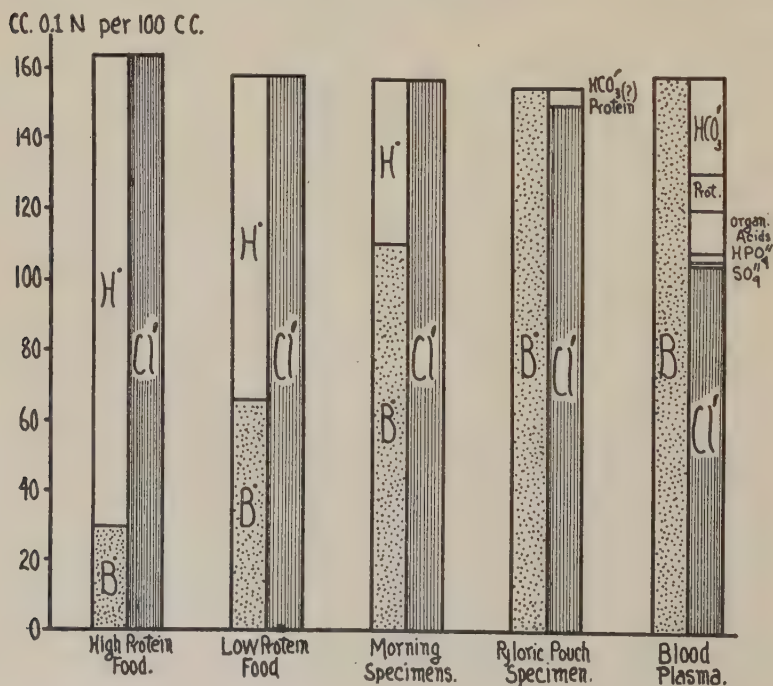
Measurements are per 100 cc. of juice.

^a Beaumont, W., *The Physiology of Digestion*, 1833.

per 100 cc.; and for a similar large specimen of juice produced following ingestion of food was 0.103 gm. per 100 cc. In these specimens only minute amounts of Ca^{++} and of PO_4''' (1.22 and 0.24 cc. of 0.1 N per 100 cc. respectively) were found. So that the values for $\text{Cl}'\text{---B'}$ in the table are probably at least approximate measurements of HCl .

From an isolated pouch in the pyloric antrum a few small samples of a thick, viscous, nearly colorless secretion were obtained by patient catheterization. The composition of this material has not as yet been thoroughly determined. Single measurements of several factors which have been obtained are, per 100 cc., as follows: B' , 155 cc. 0.1 N., Cl' , 150 cc. 0.1 N., K' , 8.8 cc. 0.1 N., HCO_3' , 5.3 cc. 0.1 N., dried substance, 2.49 gm. pH was 8.4. Apparently this secretion contains Cl' at about the same concentration as gastric juice produced in the fundus and owes its alkalinity to its much larger content of fixed base.

Referring again to the measurements of chloride ion concentration in the table, it may be noted that they are in average near the value, 158 cc. 0.1 N per 100 cc., which obtains for total fixed



base in blood plasma. ($B\cdot$) in blood plasma, owing to the adjustability of (HCO'_3) among the factors of acid equivalence, controls the total ionic content of this fluid. In gastric juice from the fundus (Cl'), other acid factors being nearly negligible and ($H\cdot$) being the result of (Cl')—($B\cdot$), should determine the total concentration of electrolytes. An approximate equivalence of total ionic content in blood plasma and in gastric juice is thus indicated.

To further illustrate this point and also to indicate clearly the size and, in contrast with the nearly stationary concentration of chloride, the mobility of the fixed base factor in gastric juice, the accompanying diagrams are presented. In them the base factors are superimposed in the left hand, and the acid factors in the right hand columns.

The measurements of fixed base and of chloride were obtained by the methods of Fiske. The procedure of Kramer and Tisdall was used in determining potassium.

3005

Parallelism between serologic and bacteriophagic response in *B. typhosus* and certain avian paratyphoids.

PHILIP HADLEY.

[From the Department of Bacteriology, University of Michigan, Ann Arbor, Michigan.]

Although not commonly recognized, there exist two curious and clearly marked discrepancies in the specific action of typhoid immune serum and typhoid antigen. In 1912 Pfeiler and Rehse¹ isolated from an epidemic of the disease, fowl typhoid, an organism which they regarded as a new species and to which they gave the name, *Bacillus typhi gallinarum alkalifaciens*. They demonstrated the agglutinability of this organism to a high titer in typhoid (human) immune serum; also a slightly reduced agglutinability of *B. typhosus* in B. T. G. A. immune serum. I have shown elsewhere² that the organism discovered by Pfeiler and

¹ Pfeiler and Rehse, *Mitteil. d. Kais. Wilhelm's Inst. f. Landwirtschaft im Bromberg*, 1912-13, v, 306-321.

² Hadley, P., *R. I. Agr. Exp. Sta. Bul.*, 1917, No. 172, pp. 1-40; No. 174, 1918, pp. 1-216.

Rehse was not a new species, but the old *B. gallinarum* of Klein, which has masqueraded under various names. In 1915 Theobald Smith and Tenbroeck³ confirmed the findings of Pfeiler and Rehse, using in this case the *Bact. sanguinarium* of Moore, which also is identical with *B. gallinarum* of Klein. In addition, they showed a similar serologic and antigenic relation between *B. typhosus* and another avian paratyphoid, the *Bact. pullorum* of Rettger, the causative agent in chick septicemia. I² have repeated the experiments of Pfeiler and Rehse, and of Smith and Tenbroeck, and have studied the serologic relationships of these avian types, not only to *B. typhosus*, but also to a number of other cultures coming from avian or mammalian sources. A resumé of the reactions usually observed, so far as they concern the subject of this paper, are presented in the following table.

TABLE I.

Showing the serologic and antigenic relationships between the organisms mentioned. Symbols: (+) positive agglutinative reaction at a high titer; (—) no agglutination; (0) no test.

Serum immune to organisms listed below	Bacterial antigens				
	<i>Bact. pullorum</i>	<i>B. galli- narum</i>	<i>B. ty- phosus</i>	<i>B. coli</i>	<i>B. dys- enteriae</i>
<i>Bact. pullorum</i> (No. 13)	+	+	+	—	—
<i>B. gallinarum</i> (No. 89)	+	+	+	—	—
<i>B. typhosus</i>	+	+	+	—	+
<i>B. coli communis</i>	—	—	—	+	0
<i>B. dysenteriae</i>	—	—	—	—	+

In view of these peculiar and easily observed antigenic relationships between *B. typhosus* and the avian paratyphoids it was a matter of interest to ascertain to what degree, if at all, similar relationships might be present in the lytic principle reactions, active and passive, between the same cultures. If the phenomena of reciprocal agglutination depend on similarities in antigenic groups, it might be assumed that these similar antigenic groups would also determine similar bacteriophagic reactions.

Accordingly I dissociated each of the five cultures concerned into the S- and R-components and worked exclusively with the freshly isolated S-types. For each of these I produced a lytic

³ Smith and Tenbroeck, *J. Med. Res.*, 1915, **xxi**, 503-521.

principle having an inhibition titer of about 1×10^{-8} when used against one standard loop of 18-hour broth culture in 10 cc. of beef infusion broth, pH 7.6. In preparing the lytic filtrates, each was carried by dilution sufficiently far from the original stock (city sewage) to insure the disappearance of any "heterologous" bacteriophage that otherwise might have passed from tube to tube in the series. These lytic filtrates were tested against the various cultures (1) by lysis on agar slants, (2) by inhibition of growth in broth. In the former tests one loop of lytic principle was streaked against 3 loops of culture according to the usual methods. In the latter tests one drop (0.04 cc.) of lytic filtrate was brought into action against one drop of 18-hour broth culture in 10 cc. of beef infusion broth. The results were usually sharp at the end of 18 hours, that is, either complete inhibition of growth or marked clouding, similar to that of the controls. After 36 hours a secondary, SR-type culture arose in nearly all the tubes showing inhibition except those containing the specific lytic agent mixed with its homologous substratum. These remained permanently clear. It is to be noted, however, that typhoid lytic principle gave complete and permanent inhibition of *Bact. pullorum*. The outcome of the tests is presented in the following table.

TABLE II.

Showing the bacteriophagic relationships between the cultures mentioned. Symbols: (++) complete and permanent inhibition; (+) complete inhibition for 36 hours; (—) no inhibition; (Cl) tube remains clear; (N) normal growth (culture controls).

Lytic filtrate for organisms listed below	Bacterial substratum					
	<i>Bact. pullorum</i>	<i>B. gallinarum</i>	<i>B. typhosus</i>	<i>B. coli</i>	<i>B. dysenteriae</i>	L. P. control
<i>Bact. pullorum</i> (No. 13)	++	+	+	—	—	C
<i>B. gallinarum</i> (No. 89)	+	++	+	—	—	C
<i>B. typhosus</i> (Eberth)	++	+	++	—	+	C
<i>B. coli</i> (Jordan)	—*	—	—	++	—	C
<i>B. dysenteriae</i> (Shiga)	—	—	±	—	++	C
Control on culture	N	N	N	N	N	

*See text.

The results tabulated above indicate that the bacteriophage reactions follow closely the trend of the agglutinative reactions presented in Table I. The only exceptions were that the lytic

principle for *B. coli* occasionally gave a slight inhibition of *Bact. pullorum* in broth; also that the Shiga lytic principle sometimes gave temporary inhibition of *B. typhosus*.

We may conclude from these observations that a bacteriophage, evolved at the expense of an organism common to the intestinal tract of man, but absent from birds, may possess *at once and in full strength* (*i. e.*, without the need of "adaptation"), power to cause complete inhibition and lysis of organisms common to the intestinal tract of birds but, so far as I am aware, never found in the human intestine. Moreover, that there exists a curious parallel between the eccentric serologic and antigenic characteristics of *B. typhosus* and its mode of response in reactions, active and passive, to the bacteriophage. It is obvious that these results are difficult to reconcile with the ultravirus theory of the nature of the bacteriophage as maintained by d'Herelle.

3006

Injections of spermatozoa and testicular substance into male rats.

WM. J. QUICK. (Introduced by A. J. Carlson).

[From the Hull Zoological Laboratory, University of Chicago, Chicago, Ill.]

In studying possible modifying effects on spermatogenesis, young adult male rats were injected subcutaneously and intraperitoneally with (1) freshly macerated testis suspensions and (2) with a suspension of rat spermatozoa. Subsequent matings were made with all injected males and the testes were later studied histologically. Agglutination tests were not made.

It was found that one to five relatively heavy injections of these materials produced no specific effect on testicular activity within the limits of the experiment (three days to four months after the last injection). Smears from testes of previously injected males showed sperm motility, and litters obtained from matings proved the spermatozoa physiologically normal.

Formed materials injected into the abdomen at times resulted in the production of cysts or abscesses sometimes located in the scrotum. When these latter were sufficiently large to cause displacement of the testis into the abdomen, the testis was found to be degenerate. Such degeneration, however, was not due to serological influences of the injected materials, but solely to the mechanical displacement of the organ; removed from the scrotal regulatory influences degeneration of the testis follows rapidly. In the abdomen degeneration is brought about through the exposure to higher than normal temperature for the testis and this degeneration should be carefully distinguished from supposed serological influences.

3007

Observations on the growth of yeasts in pure nutrient solutions.

F. W. TANNER and LOUISE MILLHOUSE.

[*From the Department of Bacteriology, University of Illinois, Urbana, Ill.*]

The multiplication of yeasts in pure nutrient solutions has already received much study; as a result there are many discrepancies in conclusions which have been reached. This brief report deals with two phases of the question: 1, the effect of concentration of an accessory containing substance, and 2, the effect of aeration on the multiplication of yeasts in pure nutrient solutions.

In both of these studies, Fulmer and Nelson's Medium E¹ was used. To study the first part of the problem Fred, Peterson² and Davenport's yeast water medium² was added to medium E in different dilutions, as follows: 1, 100 cc. medium E + 10 cc. sterile water; 2, 100 cc. medium E + 10 cc. yeast water medium; 3, 100 cc. medium E + 10 cc. of a 1-10,000 dilution of yeast medium, and 5, 100 cc. of a medium E + 10 cc. of a

¹ Fulmer, E. I., and Nelson, V. E., *J. Inf. Disc.*, 1923, xxxiii, 130.

² Fred, E. B., Anderson, W. H., and Davenport, A., *J. Biol. Chem.*, 1920, xlii, 175-189.

1-1,000,000 dilution of yeast medium. These cultures after sterilization and inoculation were incubated under the same conditions. *Saccharomyces anomolous*, *Torula colliculosae*, *Willia saturnus*, *Zygosaccharomyces mandschuricus* and *Pichia farinosus* were used.

The results showed that when the yeast water medium was present in greatest amounts there was an acceleration in multiplication, but when it was added only in minute traces, there was little difference between these flasks and the control. There was no alteration in multiplication accelerating effect by heating the yeast medium for 60 hours at 15 lbs. pressure (121.5 °C). It is believed that the acceleration in multiplication observed when large amounts of yeast water were added was due to chemically definite food substances and that one need not place the explanation on the presence of "bios." This position seems reasonable because dilution of the accessory containing substance lessened its effect on multiplication and also by the fact that prolonged heating did not alter the value of the yeast water medium.

The second part of the work was to determine whether aeration had any effect on multiplication of yeasts in pure nutrient solutions. Aeration was found to have a decided effect, the multiplication rate being many times greater in the aerated than that in the unaerated medium. For example, *Pichia farinosus* showed a maximum growth of 126 million cells per cc. in the aerated medium and only 17 million cells in the unaerated medium. These results are in agreement with the conclusions of Slator,³ who worked with beer wort. The different yeasts showed different maximums. For example, *Saccharomyces anomolous* attained a maximum of 149 million cells per cc. in the aerated medium and 34 million cells in the unaerated culture, while *Torula colliculosae* attained but 50 million cells in the aerated and 4.5 million cells per cc. in the unaerated culture. These differences are no doubt due to different nutritional requirements. Medium E seemed to be a better medium for *Saccharomyces anomolous* than for *Torula colliculosae*. It was also noticed that with all of the yeasts but one there was a period of 4 or 5 days during which the yeast did not multiply rapidly. This bears out Slator's findings for yeast in wort. The reason for the beneficial effects of aeration are problematical. It is believed that removal of CO₂

³ Slator, A., *J. Chem. Soc.*, Pt. 1, 1921, exix, 115-131.

is the most tenable explanation, although there may also have been beneficial effects from the agitation which the yeasts were subjected to by aeration. These data indicate that the incubation of cultures of yeasts under certain conditions and in vessels of certain shapes, which allow different amounts of oxygen to enter, may greatly influence the crop.

3008

Effect of sodium benzoate upon certain yeasts.

FRED W. TANNER and LUELLA B. STRAUCH.

[From the Department of Bacteriology, University of Illinois, Urbana, Ill.]

The data collected under the conditions of these experiments indicate a marked difference in resistance of pure yeasts to sodium benzoate. On dextrose agar and in dextrose broth even in the presence of 0.40 per cent of sodium benzoate, growth was appreciable. *Torula communis* did not grow after the concentration of the benzoate in the broth had reached 0.15 per cent. One strain of *Sacch. ellipsoideus* was also inhibited at this concentration. Another strain showed a greater resistance; it grew in the presence of even 1 per cent but the growth was slower and less abundant. The fungi used seemed to be able to tolerate the sodium benzoate better when growing on a solid medium containing it than when growing in a liquid medium. This is probably explained by the fact that on the solid medium the organisms may use up the disinfectant during the early stages of growth so that the cells which develop later in the history of the culture do not have to endure such a high concentration as in the beginning. In liquid media such as dextrose broth, the sodium benzoate is in solution and therefore may come into closer contact with the cells. Two of the strains of budding fungi, *Mycoderma vini* and a pure culture from vinegar (No. 18) were able to grow apparently as well in the presence of one per cent of sodium benzoate as in the controls.

In sterilized apple juice the results were more striking. The

sterile apple juice was almost as good a medium for the yeasts as the sterile dextrose broth since in the control tubes without any preservative the growth was abundant. However, when sodium benzoate was added the growth was markedly inhibited since none of the fungi grew after 0.01 per cent had been added. The same was also true for the amount of alcohol formed. These results agree with those of Scott and Will.²

Attempts were also made to study the preserving effect of sodium benzoate in catsup. No growth of the yeasts could be secured even in control tubes containing no sodium benzoate.

The above data were collected by means of pure cultures under laboratory conditions. The most important fact brought out, perhaps, is the marked difference among yeasts in resistance to sodium benzoate; also, the fact that the chemical constitution of the material to be preserved has great influence is indicated. This probably explains the greater preserving action of sodium benzoate in apple juice than in broth. It is also reasonable to assume that the species of apple from which the juice has been prepared would have considerable influence.

Preservation of apple juice with sodium benzoate seems to be a relative matter anyway. Sodium benzoate is not an absolute preservative. It may delay the fermentation for a time and in conjunction with low temperatures may exhibit a moderate efficiency. However, fresh apple juice to which sodium benzoate has been added to the extent of 0.1 per cent, according to law, will ferment. Satisfactory vinegar has been made from such apple juice. Rice and Markley¹ stated that when the yeast has had a start or if the cider is not cooled, as much as 0.5 per cent of sodium benzoate would be necessary. Scott and Will,² who worked with unsterilized apple juice reported that sodium benzoate in amounts as low as 0.02 per cent prevented the formation of an appreciable amount of alcohol. This statement is in accordance with the results secured on commercial apple juice which had been treated with 0.1 per cent (1½ oz. to 10 gallons) of the preservative.

¹ Rice, F. E., and Markley, A. L., *Bull. Ag. Exp. Sta.*, Cornell Univ., 1921, xliv, 1-23.

² Scott, R. C., and Will, E. G., *J. Indus. Eng. Chem.*, 1921, xiii, 1141-1143.

3009

Bacterial proteins.*

**WILLIAM H. WELKER, WILLIAM F. PETERSEN, P. W. RUSH and
D. M. MacCORNACK.**

[From the Laboratory of Physiological Chemistry and the Department of Bacteriology and Pathology, College of Medicine, University of Illinois, Chicago, Ill.]

The object of this work was the study of composition of bacteria so far as the proteins are concerned. It seemed to us of considerable importance to know the character of the proteins that exist in micro organisms, and further it was hoped to get definite information concerning the relative toxicity of these proteins. One of us (P) had available a supply of dried bacteria, which we had hoped would suffice for this study. Preliminary work on these dried bacteria showed the amounts on hand to be inadequate.

The first steps in the process consisted of the preparation of large amounts of bacteria. Three types of bacteria were used in this study, namely, the colon, pyocaneus, mucosa capsulatus. They were grown in pure culture on broth and were removed from the broth by centrifugalization in a Sharpless centrifuge. They were suspended in physiological salt solution and again thrown out by the use of the centrifuge, and the process repeated until they were washed free from the broth. They were then spread in thin layers on glass plates and dried.

A number of different methods were tried to disrupt bacteria, among them placing the bacteria under high pressure, then suddenly releasing this pressure, and grinding in a small glass ball mill, neither of these methods proved very successful. The method finally adopted was the grinding of the bacteria in a large porcelain ball mill in paste form with toluol for 48 hours, and subsequently with a solvent for another 48 hours. The resultant mixture was centrifugilized. The supernatant liquid was saturated to 33 per cent with ammonium sulphate. The filtrate from this precipitate was raised to 46 per cent, saturation with ammonium sulphate, filtered, and the filtrate raised to 64 per cent satu-

* Grant 52 American Medical Association.

ration. The filtrate from this was saturated with solid ammonium sulphate. The second extraction was made with the first solvent to remove the major portion of any water soluble protein that remained after the first extraction. The quantities obtained on the second extraction were always small in amount.

The residue from the centrifugalization was extracted for 48 hours in the ball mill with 10 per cent sodium chloride solution. After centrifugalization this extract was treated with ammonium sulphate in the same fashion as the water extract. A second extract was made with sodium chloride.

The next solvent used was 0.5 per cent sodium carbonate with which the residue was extracted for 48 hours in the ball mill. This was treated as the previous extracts. By this method we obtained evidence of the presence of albumin, globulin and alkali soluble protein. The amounts obtained, however, were not sufficient toxicity tests as shown by the negative results of our experiments with the available material.

In order to obtain sufficient material for toxicity tests a larger portion of the dried colon material was treated in the fashion indicated in the ball mill for the extraction of the water soluble protein. The following tests were made on this protein. After being dried this material was not completely soluble. The figures show the amount of soluble material in the solution used.

TOXICITY EXPERIMENTS.

Guinea pigs were used in these experiments. They each weighed around 250 gm.

I.

Dried Colon.

- 2.5 mg. suspended in .5 cc.—0.9 per cent NaCl—Sick.
- 5 mg. suspended in 1 cc.—0.9 per cent NaCl—Died 9½ hours later.
- 7.5 mg. suspended in 1.5 cc.—0.9 per cent NaCl—Died 5 hours later.
- 10 mg. suspended in 0.2 cc.—0.9 per cent NaCl—Died 6 hours later.
- 15 mg. suspended in 3 cc.—0.9 per cent NaCl—Died 5 hours later.
- 20 mg. suspended in 4 cc.—0.9 per cent NaCl—Died 3 hours later.

Colon Protein.

- .22 mg. dissolved in 0.1 cc.—0.9 per cent NaCl—No effect.
- .44 mg. dissolved in 0.2 cc.—0.9 per cent NaCl—No effect.
- 1.1 mg. dissolved in 0.5 cc.—0.9 per cent NaCl—No effect.
- 3.3 mg. dissolved in 1.5 cc.—0.9 per cent NaCl—No effect.
- 4.4 mg. dissolved in 2 cc.—0.9 per cent NaCl—Died 5 hours later.
- 6.6 mg. dissolved in 3 cc.—0.9 per cent NaCl—Died 5 hours later.
- 8.8 mg. dissolved in 4 cc.—0.9 per cent NaCl—Died 4 hours later.
- 13.2 mg. dissolved in 6 cc.—0.9 per cent NaCl—Died 2½ hours later.

II.

Colon Protein.

- .75 mg. dissolved in 0.5 cc.—0.9 per cent NaCl—No effect.
- 1.5 mg. dissolved in 1 cc.—0.9 per cent NaCl—No effect.
- 2.25 mg. dissolved in 1.5 cc.—0.9 per cent NaCl—No effect.
- 3 mg. dissolved in 2 cc.—0.9 per cent NaCl—No effect.

- 4.5 cc. dissolved in 3 cc.—0.9 per cent NaCl—No effect.
 6 mg. dissolved in 4 cc.—0.9 per cent NaCl—Died several hours later.

III.

- .16 mg. dissolved in .2 cc.—0.9 per cent NaCl—No effect.
 .4 mg. dissolved in 5 cc.—0.9 per cent NaCl—No effect.
 .8 mg. dissolved in 1 cc.—0.9 per cent NaCl—No effect.
 1.2 mg. dissolved in 1.5 cc.—0.9 per cent NaCl—No effect.
 1.6 mg. dissolved in 2 cc.—0.9 per cent NaCl—Died at the end of 48 hrs.
 2.4 mg. dissolved in 3 cc.—0.9 per cent NaCl—No effect.
 2.8 mg. dissolved in 3.5 cc.—0.9 per cent NaCl—Died at the end of 46 hrs.

CONCLUSIONS.

Our experiments show the presence of albumin globulin and alkali soluble protein in the colon.

The toxicity of the water soluble protein shows no marked deviation from that of the colon itself.

The work on the colon bacterial proteins will be continued on much larger amounts of the dried organism.

3010

**The influence of posture of the neck on progression of the fowl
 (*Gallus domesticus*).**

THEODORE KOPPANYI and NATHANIEL KLEITMAN.

[*From the Hull Physiological Laboratory of the University of Chicago, Chicago, Ill.*]

In the course of our investigation of the body righting reflexes in the fowl, a detailed report of which will appear shortly, we had one animal which as a result of a cranial operation assumed a peculiar posture at certain times. It would keep its head down and tail up, so that the long axis of the body was at an angle about 60° to the horizontal. When in such a condition, the animal invariably walked backward and continued to do so till it would hit the wall of the room. But when it kept its head in a normal position it could and did walk forward.

In order to find out the mechanism of this phenomenon we studied a number of normal Leghorn and Sebright Bantam fowls in which the lower portion of the neck was kept flexed upon the trunk by means of a gauze bandage. We found that these ani-

mals, without exception, in attempting to move walked backward. After the bandage was removed they immediately returned to the normal method of progression. Such test could be performed repeatedly on the same animal with similar results.

To determine whether this retrogression was not due to voluntary movements of the nature of escape reaction, we performed these tests on the decerebrated fowl. The results were the same as in normal animals, indicating that this phenomenon is of a reflex nature (forced movement).

We conclude, therefore, that normal progression in the chicken is dependent upon the posture of the neck, or the tonic state of the neck muscles. This ability is lost as soon as the posture of the neck is changed (neck ventroflexed upon the trunk), and progression is replaced by retrogression.

3011

On the existence of a pressor substance in the blood of clinical cases of hypertension.

C. S. DANZER, J. G. BRODY and A. L. MILES.

*[From the Sections of Experimental Medicine and Physiology,
New York Homeopathic Medical College and
Flower Hospital, New York City.]*

It is common knowledge that neither the etiology nor the pathogenesis of hypertension is understood. In a rather loose way it has been correlated with diseases of the arteries and kidneys. Whether the reduction in the cross sectional area of the vascular bed is due to anatomical changes in these structures, or to some chemical substance circulating in the blood, which is capable of exerting a pressor response, has never been answered adequately.

We have, therefore, attacked the problem by transfusing the unchanged blood of patients with hypertension into animals, in order to see whether a pressor response could be detected in the animals so treated. In such a case the theory of the reduction of the peripheral vascular bed as the primary cause of hypertension could be excluded. Likewise the hypothesis of Hülse¹ regard-

¹ Hülse, *Z. ges. Exp. Med.*, 1922, xxx, 240 and 268.

ing vascular hyperirritability would have to enter into serious consideration in the explanation of the genesis of high blood pressure.

The search for pressor substances in the blood is by no means new. Very soon after the development of the studies on epinephrin the idea naturally suggested itself that this substance which exists in the blood normally, because of its blood pressure raising influence, would be found in excess in cases of hypertension. This seemed particularly likely in view of the pathological reports that medullary hypertrophy or tumors of the suprarenal gland had been found in association with hypertension. That these findings were only chance occurrences was proved by their presence without hypertension, and conversely, hypertension without suprarenal hyperplasia. Bröking and Trendelenburg,² and Stewart³ tested for adrenalin in cases of hypertension with negative results. They admit the crudeness of the methods used for the detection of these substances.

Exper. 1.—Varying amounts of physiological saline, from two to twenty cc. were injected into the veins of a cat. In each case a small rise of pressure occurred. The effects on blood pressure, of the smallest and the largest dose, were equal.

Exper. 2.—20 cc. of homologous fresh blood were transfused into a cat. The immediate, small rise in blood pressure was similar to that resulting from the injection of the same amount of saline.

Exper. 3.—12 cc. of rabbits' whole blood transfused into a cat produced the usual initial volume effect on the blood pressure followed by a marked fall. This depressor effect will be explained later.

Exper. 4 and 5.—12 and 16 cc. of unchanged human blood taken from two women with normal arterial pressures were transfused into two separate cats. In each case there resulted the slight initial rise due to the volume injected.

Exper. 6.—20 cc. of human blood from a case of hypertension were transfused into an animal (cat). The usual short primary rise was followed by a prolonged great secondary rise in the mean, systolic, diastolic and pulse pressure, together with a slowing of the heart rate.

² Bröking and Trendelenburg, *Deutsche Arch. f. klin. Med.*, 1911, ciii, 168.

³ Stewart, *J. Exp. Med.*, 1911, xiv, 377.

Exper. 7.—In another cat the injection of 18 cc. of the same patient's blood produced the same initial rise in blood pressure. The secondary effect was the mirror image of the first one—a marked dropping in pressure with cardiac slowing resulted. This depressor and bradycardic effects were only to be interpreted after the subsequent experiments were performed.

Exper. 8.—18 cc. of whole blood from a clinical case of hypertension was transfused into a cat. The insignificant primary rise followed by a marked fall in pressure, accompanied with a slow large pulse pressure oscillations, followed. This secondary effect is clearly of vagus origin as was shown in the next experiment, No. 9, where the same procedure was carried out on an atropinized animal. Here there was no depressor effect. In the following experiment, No. 10, which was a repetition of 9, atropine not only prevented the secondary drop in blood pressure, but also allowed the secondary pressor effect to make itself manifest. In the latter case there was no increase in pulse pressure.

Exper. 11.—12 cc. blood of another woman with hypertension were transfused into a unatropinized cat. There occurred a very marked, prolonged rise, followed by a very pronounced fall in blood pressure and disappearance of pulse pressure, both of which gradually rose and were later followed by a bradycardia. Here again we see a pressor effect, followed by a depressor (Vagus) phase.

Exper. 12.—An alcoholic extract of the blood of a hypertensive (case used in Exper. 8-9-10) was made, dissolved in water. Ten cc. were injected into the vein of a cat whose vagi were unaffected. An immediate drop in pressure and slowing of the heart occurred. The depressor effect was so powerful that it overshadowed even the primary volume effect. When this extract was diluted five times and 10 cc. infused into the cat's vein (Exper. 13) both a primary and secondary rise of blood pressure occurred very similar to the effect of unchanged blood from the same patient.

It is obvious that we are dealing in the blood mixture with two substances, a depressor substance which was present in most of the cases of heterologous blood injected, and a pressor substance. That the former was probably protein in nature, and the reaction was anaphylactic in character, seemed quite probable.

To test this 1 cc. of horse serum was injected into the vein of

a cat and produced the expected depressor and bradycardic (Vagus) effect (Exper. 14).

In Exper. 15 an attempt was made to desensitize the cat against the specific protein (horse-serum) by injecting the latter, diluted and in small amounts, into the vein before the large amount of concentrated protein was given. The latter then had only a very slight depressor effect, which would not interfere seriously with our experiments. The animal could in a similar manner be desensitized against an alcoholic extract of rabbits' blood. (Exper. 16).

Exper. 17.—Cat was vagotomized on both sides to eliminate the vagus effect and desensitized in the usual way against human blood from a clinical case of hypertension (same case as in Exper. 8-9-10). Now 18 cc. of unchanged blood of this patient were transfused into the cat. A negligible primary effect but a very striking secondary effect occurred. This had the usual delay, but the pressor effect was greater than in any of our previous experiments. Apparently the method of desensitization and vagus exclusion allowed the pressor effect to unfold itself in bold relief.

Exper. 18.—Eighteen cc. of the same blood were injected into a cat with intact vagi but desensitized by the previous injection of 4 cc. of the same blood subjectaneously. A very pronounced primary rise in blood pressure occurred. This curve was checked against that produced by varying amounts of epinephrin, and corresponded very closely with the curve obtained from the injection of .5 cc. epinephrin 1-1000 dissolved in 19.5 cc. of normal saline. The pressor substance may thus be expressed in terms of epinephrin units.

Exper. 19.—10 cc. of blood cells of a case of hypertension were injected into the vein of a cat and a delayed though marked pressor effect was observed.

CONCLUSIONS.

1. Blood from cases of hypertension contains a pressor substance.
2. The blood proteins by virtue of their anaphylactic reaction and the intact vagi of the experimental animals inhibit the otherwise obvious pressor response. Hence these factors should be eliminated in such experiments.
3. No pressor effect of any kind was ever seen by us from the injections of comparable amounts of saline, rabbits' blood or human blood of people with normal blood pressures.

Western New York Branch

University of Buffalo, February 13, 1926.

3012

Digestion and absorption of cereal proteins in the human alimentary tract.

J. R. MURLIN, H. A. MATTILL, J. S. CARMAN and ELSIE M. AUSTIN.

[From the Department of Vital Economics, University of Rochester, Rochester, N. Y.]

Rapidity of digestion of the protein contained in different cereal breakfast foods was determined in human subjects, partly by the emptying time of the stomach as measured by the total nitrogen in its contents obtained at successive intervals after a cereal breakfast, and partly by the rate of nitrogen excretion through the kidney following the meal. The former method obviously gives information chiefly upon the motility of the stomach.

Each of four subjects ingested 25 grams of dry cereal, after cooking a uniform length of time, with cream and sugar and in some cases coffee. Before the meal a Rehfuß tube was introduced and the fact established that the stomach was empty. The tube was left in place and one hour after the meal the contents of the stomach were completely removed by the use of large amounts of wash water. Each subject repeated the experiment on the following morning with the same cereal and with exactly similar technique, except that the stomach was emptied at one and a half hours. On succeeding mornings the time was 2 hours, 2½ hours, etc., until the stomach was empty. The next week each subject took a different cereal with another change for the third, and still another for the fourth week. The cereals employed were a milled wheat product, a whole wheat product and two oats products.

The differences in stomach emptying time amongst the four cereals were small, much less than those found amongst the dif-

ferent subjects for the same cereal. An average of all the subjects for all the cereals indicated that at the end of one hour 35 to 45 per cent of the ingested cereal protein had passed out of the stomach, at two hours about 75 per cent and at three hours the average stomach was empty. Oats protein tended to leave more rapidly during the early part of digestion but was delayed in the later part.

Reliable data on rate of absorption were obtained from two subjects in connection with work and dynamic action experiments. The nitrogen of the milled wheat product regularly appeared sooner than the nitrogen from ingested oats. This was confirmed for the carbohydrate of the cereals by its effects upon the respiratory quotient.

Figures on the extent to which the cereal proteins are absorbed in the entire alimentary tract were obtained from experiments in which the fecal nitrogen and its metabolic fraction were known. From these data and the figures for ingested nitrogen the percentage absorption or utilization of the cereals are as follows: Milled wheat product 99.5 per cent, whole wheat product 87.2 per cent, oat product 86.7 per cent. These figures apparently verify the generally accepted opinion that roughage, necessary as it is, decreases the percentage of utilization.

3013

The rate of glycogen formation in the liver during absorption of fructose and galactose.

CARL F. CORI.

[From the State Institute for the Study of Malignant Diseases, Buffalo, N. Y.]

The rate of glycogen formation in the liver of the rat during the absorption of glucose from the intestine, and the influence of insulin on the rate of glycogen formation has been studied on a former occasion.¹ It seemed of interest to repeat these experiments during the absorption of fructose and galactose.

¹ PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 286.

EXPERIMENTAL.

The method used has been described in our previous publication.¹ Five male rats were killed 1, 2, 3, 4, and 5 hours after feeding fructose by stomach tube. The amount of sugar absorbed from the intestine and the amount of liver glycogen formed was determined on each animal. The average amounts of glycogen formed per 100 gm. liver were as follows: After 1 hour, 0.48 gm.; after 2 hours, 1.60 gm.; after 3 hours, 2.79 gm.; after 4 hours, 3.95 gm.; and after 5 hours, 4.23 gm. If these values are represented in the form of a curve and compared with the curve that has already been published for glucose, it will be noted that the curve for fructose shows a steeper rise and reaches a higher glycogen maximum than the curve for glucose.

If one calculates the percentage of the total amount of sugar absorbed that is retained in the liver in the form of glycogen, this exceeds, as an average, 30 per cent for the 2, 3, 4, and 5 hour absorption periods. In some individual experiments as much as 45 or even 50 per cent of the absorbed fructose was converted into liver glycogen. This is a surprisingly large amount if one considers that the liver of rats fasted for 48 hours constitutes only 3 per cent of the body weight. In the former experiments with glucose¹ only 14 to 18 per cent of the absorbed glucose was built up into liver glycogen. The lower percentage of glucose that is retained as liver glycogen is mainly due to the fact that glucose is absorbed twice as fast as fructose. The liver, from a quantitative standpoint, plays a much more important role in removing fructose from the blood stream than glucose. There is also evidence that the kidney threshold for fructose is very low. These two factors might explain why the tolerance for ingested fructose has been found a suitable test for liver function.

Insulin has a stronger inhibiting influence on the rate of glycogen formation from fructose than from glucose. In the first three of fructose absorption no glycogen is formed, when 10 to 15 units per 100 gm. of body weight are injected simultaneously with the sugar feeding. After 4 or 5 hours a small amount of glycogen is deposited. Hypoglycemic symptoms were absent in these animals. During the absorption of glucose glycogen was already formed in the second hour of insulin action.

The experiments with galactose indicate that this sugar is converted very slowly into glycogen, and that a large part of this sugar is excreted in the urine. The average amounts of glycogen

formed per 100 gm. liver were as follows: After 2 hours, 0.52 gm.; after 3 hours, 0.68 gm.; after 4 hours, 1.36 gm. The excretion of galactose in the urine shows many striking features and needs further investigation. The percentage of the absorbed galactose that is excreted increases more and more the longer the absorption proceeds, in spite of the fact that the rate of absorption remains constant. Thus, in one hour 27 per cent of the absorbed galactose appears in the urine, in two hours 41 per cent, in three hours 51 per cent and in four hours 60.5 per cent.

SUMMARY.

1. The rate of glycogen formation in the liver during the absorption of fructose is slightly greater than during the absorption of glucose and leads to a higher glycogen maximum.
2. The liver plays a larger role in removing fructose from the blood stream than glucose.
3. Large doses of insulin almost completely suppress the glycogen formation from fructose.
4. Galactose is very slowly converted into glycogen and is excreted to a large extent in the urine.

3014

The influence of insulin on the tolerance for intravenously injected glucose and fructose.

GERTY T. CORI and CARL F. CORI.

[*From the State Institute for the Study of Malignant Diseases, Buffalo, N. Y.*]

It has been found previously¹ that non-fasting male and female rats during amytal narcosis show no glycosuria, when glucose is infused at a rate between 2.2 and 2.5 gm. per kilogram per hour. Woodyatt's² figure for the intravenous tolerance limit of rabbits, dogs and men was only 0.85 gm. glucose per kilogram per hour.

¹ PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 127.

² Woodyatt, R. T., Sansum, W. D., and Miller, R. M., *J. Am. Med. Assn.*, 1915, lxx, 2067.

It seemed, therefore, of interest to investigate whether insulin would be able to raise the high intravenous tolerance of rats. It has also been shown that the maximum rate at which glucose could be absorbed from the intestine was below the maximum rate at which glucose could be infused intravenously without causing glycosuria. It seemed desirable to study if this was also true for fructose.

EXPERIMENTAL.

The method used has been fully described in our previous paper.¹ The infusions were again made into the femoral vein and were extended from 1 to 3 hours. For the present experiments only male animals were used. They were fasted for 48 hours previously. The preliminary fasting period did not change the intravenous tolerance for glucose to any larger extent, as can be seen from the following data: Four rats received glucose at a rate of 2.2, 2.4, 2.5, and 2.5 gm. per kilogram per hour and showed no glycosuria, while 2 rats which were infused at a rate of 2.6 gm. per kilogram per hour, excreted sugar in the urine.

Insulin, in doses varying from 20 to 40 units, was injected subcutaneously 30 to 45 minutes previous to the start of the infusion, and the injection was repeated at the beginning of each new hourly infusion period. In 5 experiments with insulin a rate of infusion of 2.7, 2.9, 3.0, 3.0, and 3.4 gm. glucose per kilogram per hour failed to produce glycosuria. In 8 other experiments a rate of infusion of 3.0, 3.1, 3.2, 3.2, 3.2, 3.2, 3.4, and 3.6 gm. glucose per kilogram per hour was above the tolerance.

These data indicate that the intravenous tolerance of rats which received large doses of insulin is close to 3.0 gm. glucose per kilogram per hour. It will be noted that the effect of insulin is very slight. There is obviously a limit to the amount of glucose that can be metabolized in the body. Rats, with the aid of their own insulin production, almost reach this limit and, therefore, the injection of insulin from without does not raise the tolerance very markedly. Burn and Dale³ used decapitated cats from which all the abdominal organs including the pancreas had been removed. From the amount of glucose that had to be infused intravenously in order to prevent the blood sugar from falling, they calculated the amount of sugar removed. This was, without insulin, approximately 0.15 to 0.2 gm. per kilogram per hour. The greatest observed rate of sugar disappearance during insulin

³ Burns, J. H., and Dale, H. H., *J. Physiol.*, 1924, lix, 164.

action was approximately 0.8 gm. per kilogram per hour. The low initial sugar tolerance would indicate that the preparations were in a diabetic condition and hence the strong effect of insulin is not surprising.

The experiments with fructose showed that the intravenous tolerance limit was at a rate of 0.35 gm. per kilogram per hour. Insulin injections had no decided influence on the intravenous tolerance for fructose. The quantitative estimations of fructose in the urine were checked in most instances by the Seliwanoff reaction. The rate of absorption of fructose from the intestine was found to be close to 0.8 gm. per kilogram per hour.⁴ Fructose was not excreted in the urine even if the absorption of this sugar from the intestine was allowed to proceed for 5 hours. Obviously, the intravenous tolerance for fructose is markedly below the rate at which this sugar is absorbed from the intestine. How could this discrepancy of the tolerance, when tested by the intravenous route and by the intestinal route be explained? There is evidence that the kidney threshold for fructose is very low. If fructose is absorbed from the intestine, it passes first through the liver, before it reaches the general circulation. The liver plays a very important role in removing fructose from the circulation, as is set forth in the preceding paper. When fructose is infused into the femoral vein, the liver cannot quickly enough intercept this sugar and the fructose concentration in the blood might easily rise above the kidney threshold. In order to test this possibility, fructose was infused into a mesenteric vein. In 6 rats the tolerance by this route was close to 0.7 gm. per kilogram per hour or almost as high as the rate of absorption of fructose from the intestine.

SUMMARY.

1. The intravenous tolerance of male rats, fasted previously for 48 hours, was close to 2.5 gm. glucose per kilogram per hour.
2. Large doses of insulin raised the tolerance to 3.0 gm. glucose per kilogram per hour.
3. The tolerance for fructose, when infused into the femoral vein, was close to 0.35 gm. per kilogram per hour.
4. The tolerance for fructose, when infused into a mesenteric vein, was between 0.6 and 0.8 gm. per kilogram per hour.
5. Insulin had practically no effect on the intravenous tolerance for fructose.

⁴ *J. Biol. Chem.*, 1925, lxxi, 691.

3015

Seasonal periodicity in man: I. Basal metabolism; respiration; cardio vascular condition.

FRED R. GRIFFITH, JR., G. W. PUCHER, J. D. KLEIN and M. E. CARMER.

[From the Department of Physiology, University of Buffalo, Buffalo, N. Y.]

Four normal persons, two men and two women, have served as subjects for this investigation. On each, the following observations were made once a week throughout the year (February, 1925—February, 1926) under basal conditions between 8 and 10 a. m. The observations and their apparent course throughout the year follow:

1. Oral temperature; no certain periodicity.
2. Reclining systolic pressure: all show a marked depression in the spring and in three cases there are also low points in mid-summer and late fall—early winter.
3. Reclining pulse: lowest in summer.
4. Alveolar air:
 - (1) Oxygen, uncertain, with perhaps a tendency to be lowest in summer;
 - (2) Carbon dioxid, highest in the summer in all subjects and rhythmically coincident with the menstrual cycle in the women, the low points coming just before the onset of menstruation.
5. Pulmonary ventilation: both the tidal and minute volumes show a marked depression in the spring with a rise to maximum in late summer and early fall.
6. Expired air: the percentages of oxygen and carbon dioxid vary inversely, the former showing a marked low point in spring and a maximum between July and September, while the latter is highest in the spring and lowest during late summer and early fall.
7. Carbon dioxid per minute: no evident periodicity.
8. Oxygen per minute and calories per square meter per hour: lowest in the period, July to September.
9. Respiratory Quotient (non-protein): with one exception seems to be highest in the summer and early fall.

Under the same basal conditions and either just preceding or following the above determinations 20 cc. of blood were taken from an arm vein. Most of this was used for other determinations to be reported on later, but the following may be mentioned here:

10. Blood count:

- (1) Red cells—increase to a maximum during the summer;
- (2) White cells—highest in the spring and lowest during the summer;
- (3) Differential white count—no evident periodicity.

11. Blood gas capacity:

- (1) Carbon dioxide—maximum in spring and fall with low points between;
- (2) Oxygen—maximum during summer.

Immediately following the above determinations, which were made while the subject was reclining (including the taking of the blood sample), the subject arose and served for the following additional determinations:

12. Standing pulse rate and systolic blood pressure and the pulse rate after a standard exercise: these values show no evident seasonal periodicity common to all subjects, though each one shows large and somewhat rhythmical fluctuations; the same applies to the cardio-vascular rating of Schneider, which is computed from them.

13. Vital Capacity: there seems to be a low point in the early summer and spring and a maximum in the fall.

14. Weight (Stripped): shows no periodicity.

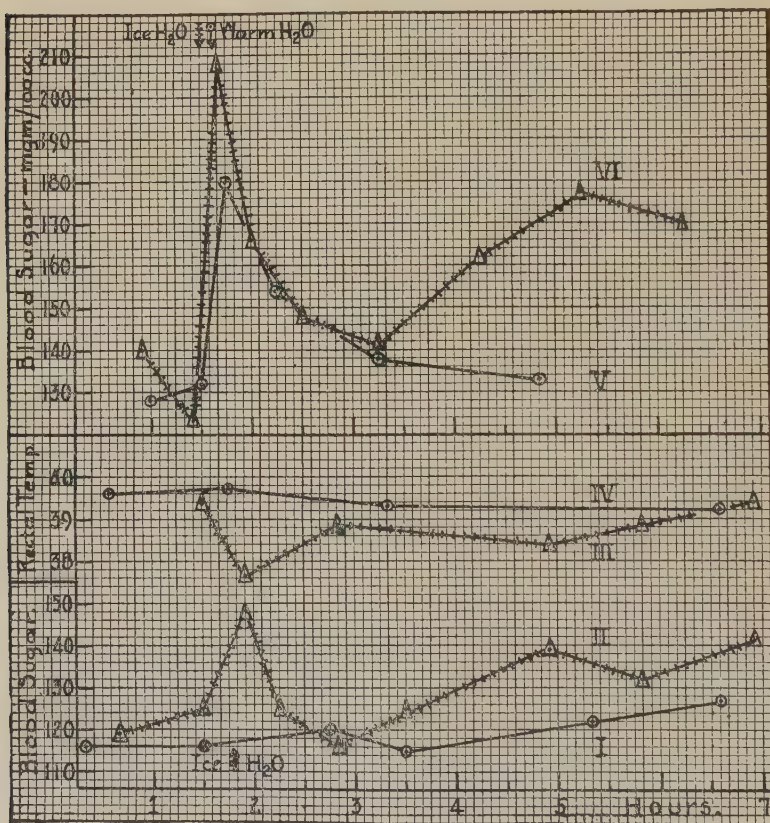
As was indicated above, enough blood was taken from each subject under basal conditions to allow a rather complete blood analysis. Each subject also collected for analysis a twenty-four hour urine on the preceding day. The results of the blood and urine analyses will be reported at a later date.

The work is being carried through a second year; the above results should, therefore, be accepted only tentatively until the additional data are at hand.

Carbohydrate mobilization in body temperature regulation.

FRED R. GRIFFITH, JR.

[From the Department of Physiology, University of Buffalo, Buffalo, N. Y.]



Cat, ♂, wt. 3.4 Kg.

I and IV, the normal fluctuations in blood sugar and body (rectal) temperature, respectively, throughout the usual duration of these experiments. (March 4, 1925.)

V, the effect of 200 cc. warm water (temp. 38-39° C.) on the blood sugar value (Dec. 18, 1924). This serves as a control for the effect of the excitement and struggling attendant on passage of the stomach tube.

II and III, the effect on the blood sugar and body (rectal) temperature, respectively, of 200 cc. ice water (0.5-1.0° C.) by stomach tube (Feb. 28, 1925).

VI, the effect on the blood sugar level of 200 cc. ice water by stomach tube, Dec. 11, 1924.

This is to report the effect on the blood sugar level of cats of a "heat liability," produced by giving ice water by stomach tube, according to the method devised by Cannon. Eight animals have been used and all have given concordant results, of which those for one cat shown in the figure are typical. From inspection of the curves (and deducting the effect of the similar procedure in which water at body temperature was given), it appears that such a heat liability induces a pronounced mobilization of carbohydrates and increase of blood sugar value. In spite of the fact that the fall in body temperature is immediate, this mobilization of sugar seems to get under way slowly and is only noticeable after an hour or more. From then on it rises slowly to a maximum during the next hour or two and then, even more slowly, falls; in only two cases out of the eight did the blood sugar value return to normal within the five or six hours during which the animals were kept under observation.

3017

Experiments with adrenal insufficiency.**FRANK A. HARTMAN.**

[From the Department of Physiology, University of Buffalo, Buffalo, N. Y.]

More than two years ago we began experiments to determine whether it would be possible to prolong the life of adrenalectomized animals by the administration of adrenal preparations.

Adrenalin injected intramuscularly or subcutaneously every hour or two after the removal of both adrenals did not prolong life. The average length of life for the 7 cats studied was 19.7 hours. The amounts injected at one time were from 0.25 cc. to 0.5 cc. of 1:1000 adrenalin intramuscularly or subcutaneously. The adrenals had been removed from these cats at one operation.

The remainder of the experiments were conducted upon cats whose adrenals had been removed at different operations from two to ninety days apart. The operation was through the lumbar path.

Five cats which were injected intravenously or intramuscularly with material obtained by ether extraction of the dried cortex (beef) lived an average of sixty hours. Two cats injected intravenously or intramuscularly with glycerol extract of the cortex lived an average of forty-five hours.

Nine cats which were injected intramuscularly or intraperitoneally with alcohol soluble material from the cortex lived an average of forty hours. One animal receiving intraperitoneal injections of olive oil extract of the cortex lived ninety-three hours.

The average length of life of all of these cats (17), not including those injected with adrenalin, was 49.8 hours.

On the other hand, 22 adrenalectomized cats injected intravenously, intramuscularly, intraperitoneally or subcutaneously with whole saline extracts of cortex lived an average of 146.6 hours.

3018

Comparative reduction values of carbohydrates by Hagedorn-Jensen, Meyer-Benedict and Folin-Wu Methods.

GEORGE W. PUCHER and MYRON W. FINCH.

[From the Department of Biologic Chemistry, University of Buffalo Medical School, and the Department of Laboratories, Buffalo General Hospital, Buffalo, N. Y.]

Dutcher and Thomas,¹ Greenwald, Samet and Gross,² Williamen and Davison,³ Rowe and Wiener,⁴ have published data on the reduction value of mono and polysaccharides by several commonly employed sugar methods. None of these investigators, however, have examined the behavior of carbohydrates toward potassium ferrocyanide, the principle of the Hagedorn-Jensen method. In this paper a comparison is presented of the values obtained by this method with those obtained by employing the Lewis-Benedict and the Folin-Wu procedures.

¹ Thomas, W., and Dutcher, R. A., *J. Am. Chem. Soc.*, 1924, xlv, 1662.

² Greenwald, I., Samet, J., Gross, J., *J. Biol. Chem.*, 1925, lxii, 397.

³ Williamen, J. J., and Davison, F. R., *J. Agr. Res.*, 1924, xxviii, 479.

⁴ Rowe, A. W., and Wiener, B., *J. Am. Chem. Soc.*, 1925, xlv, 1698.

Carbohydrate	Pieric Acid Method.					Folin-Wu.			Hagedorn-Jensen	Schaefer	Lumsburing and Thomas
	Pucher	Greenwald	Rowe	Dutcher	Williamen	Pucher	Greenwald	Rowe			
Glucose	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Fructose	1.174	.96	.99	.99	1.00	1.125	.91	.90	1.187	.90	.91
Galactose	.925	.95	.85		.883	.748	.75	.77	.795	.80	
Arabinose	1.110	1.02		1.01	1.000	.866	.80		.919	.80	1.00
Xylose	1.057	1.09		1.08	1.071	1.028	.94		.918	.96	1.01
Lactose	.763	.78	.76		.750	.475	.45	.45	.655	.53	
Maltose	.743	.82	.82		.770	.411	.40	.40	.754	.42	

The carbohydrates employed were: glucose, fructose galactose xylose, arabinose, lactose and maltose. The results recorded are the average of a large number of independent series. The purity of all the sugars was checked by means of the polaroscope.

It was found that the Hagedorn-Jensen method gives the best agreeing results. The values found by us in comparison with those of other investigators are summarized in the following table:

Studies were also made to determine some of the factors which might explain the anomolous behavior of galactose toward the Folin-Wu sugar reagents.

The conclusions reached from this study are as follows:

SUMMARY.

1. Our values except those for levulose agree with the data of previous investigators.
2. Levulose has a higher reduction value than glucose.
3. The difference in behavior of galactose and glucose toward Folin-Wu copper solution is not due to difference in velocity of reduction or differences in pH, but to structural differences in the two sugars.
4. Glucose picric acid standards used in the Benedict-Lewis methods are stable for a period of at least two years.

3019

A rapid method for determination of the free and hydrolyzable sugar content of foodstuff.

GEORGE W. PUCHER.

[From the Department of Biological Chemistry, University of Buffalo Medical School, and the Department of Laboratories, Buffalo General Hospital, Buffalo, N. Y.]

One of the most important factors in the preparation of diabetic diets, as well as in the study of the relationships between diet and sugar metabolism, is the carbohydrate content of the injected foodstuffs. The most extensive data on this subject is

found in Bulletin No. 28 of the U. S. Department of Agriculture. Unfortunately the carbohydrates in the majority of cases have been determined by difference and hence do not differentiate between the free and hydrolyzable sugar content. Furthermore, since the carbohydrate content of fruits and vegetables varies between wide limits, it is often necessary to analyze the diet used, rather than depend upon figures gathered at random. Of the various procedures for the analysis of the carbohydrate content of foodstuffs the method of Dutcher and Thomas for reducing sugars in plants seemed to adapt itself most readily to the rapid analysis of food materials.

1. Dutcher and Thomas¹ method for analyzing the free sugar content of plant material has been adopted to the analysis of the free carbohydrates in general foods and fruits.

2. Lloyd's reagent may be substituted for mercuric nitrate as a clarifying agent and the Benedict-Osterburg or Lewis-Benedict methods are applicable in the presence of alcohol.

3. A four-hour extraction period with 75 per cent alcohol is sufficient to extract completely 2,000 mgs. of glucose, fructose, galactose, xylose, and arabinose.

4. Maltose, lactose, raffinose and sucrose are extracted by 75 per cent alcohol.

5. Sucrose is not appreciably hydrolyzed by a four-hour extraction with 75 per cent alcohol, nor as pointed out by Dutcher and Thomas, is it hydrolyzed by 15' boiling with picric acid and Na_2CO_3 .

6. The analysis of a few common food materials for free and hydrolyzable carbohydrates is presented.

¹ Thomas, W., and Dutcher, R. A., *J. Am. Chem. Soc.*, 1924, xlv, 1662.

Free and Hydrolyzable Sugar in Some Common Foods.

Foodstuff	Per cent free sugar	Per cent hydrolyzable sugar	Per cent total sugar	Sugar by Bull. 28, U. S. Dept. of Agriculture
Grapefruit (fresh)	3.57 4.17	1.80 2.20	4.37 6.38	
Grapefruit (canned)				
Juice	13.0	6.5	19.5	
Pulp	5.0	3.0	8.0	
Banana, yellow				
Edible portion	17.2	9.2	26.4	21.0
Cantaloupe	1.90 2.31 1.68	0 0 0	1.90 2.31 1.68	
Watermelon	2.80 3.01 4.69	0 0 0	2.80 3.01 4.69	6.7
Melon (Catauba)	2.38			
Pineapple	2.50 1.85 1.92			9.7
Raspberries				
Seedless grapes	5.94	trace		
Tokay grapes	14.52	0.46	14.98	14.4
Sour cherries	6.52	trace		
Carrots (fresh)	4.30 4.50 4.40 4.80 2.58 2.50 1.60			
Carrots (boiled)	2.08	5.95		
(4.50 before cooking)	3.70	4.10		
Beets (boiled)	2.60			
Gluten bread	less than 0.2			
Gluten bread		37.0		49.8
Washburn-Crosby	trace	14.8		49.8
Krinkles (Diabetic)				
Cereal-Curdolac Food Co., Wankeska, Wis.	none	20.0		
Washed bran	none	6.0		
Baker's Premium				
Cracked Cocoa	none	0.52		
Maltose Chocolate Candy,			35.6	
Battle Creek Food Co.			40.0	
Swedish bread, Allon				
Bergmouir, Stockholm	0.5	56.6	57.1	
Swedish Bread, Holland				
Rusk Co., Holland, Mich.	trace	48.0	48.0	
Essential Bread				
Hendeber			59.5	
Biscottes de Chatel				
Guyon			68.0	
Biscuits Croquettes				
Dr. Charasse			51.0	
Chocolate Pastells			27.0	
Kellogg's Bran	none			
Battle Creek, Mich.		39.0		
Bran crackers	trace	57.2		

3020

Preliminary report on the isolation of a crystalline ozazone from normal urine.

GEORGE W. PUCHER.

[From the Department of Biological Chemistry, University of Buffalo Medical School, and the Department of Laboratories, Buffalo General Hospital, Buffalo, N. Y.]

When normal urine is treated with phenylhydrazine a reddish compound can be isolated. This compound is non-crystalline, appearing under the microscope in the form of round masses. This substance on treatment with chloroform can be separated into two fractions, the one soluble in chloroform, an amorphous deep red powder, the other insoluble in chloroform, a light yellow compound. The latter compound crystallizes from water in clusters of short stocky needles. It also may be purified by recrystallization from 70 per cent ethyl alcohol. The ozazone melts at 150-153°C (corr.) with decomposition, and is soluble in pyridine. On treatment with acids the compound yields furfural. The substance contains nitrogen, but so far no satisfactory analyses have been obtained. It does not correspond in its properties or crystalline structure to known hexose, pentose or glucuronic acid ozazone. Further work is in progress on the identification of this compound.

Iowa Branch

State University of Iowa, February 3, 1926.

3021

The formation of urea in autolysis.

V. C. MYERS, M. RINGER and O. O. BENSON, JR.

[From the Biochemical Laboratories of the New York Post-Graduate Medical School and Hospital, New York City, and the State University of Iowa, Iowa City, Iowa.]

As a result of the recent experiments of Bollman, Mann and Magath¹ there is now little doubt that the liver plays the leading rôle in urea formation. Nevertheless very little is known concerning the mechanism of urea formation. To be sure we have long known from the experiments of Kossel and Dakin² that arginine may be split directly into urea and ornithine under the action of a specific enzyme, arginase, but relatively this accounts for only a small amount of the urea formed.

We wish to record observations of autolysis experiments carried out some years ago³ (1914) which we have recently confirmed and extended⁴. The original motive for initiating these experiments was the availability of a specific method for estimating urea (Marshall). We were able to show then that whereas urea formation took place in autolyzing liver tissue, no change in the concentration of urea occurred in muscle tissue similarly treated.

Since our first experiments were carried out somewhat simi-

¹ Bollman, J. L., Mann, F. C., and Magath, T. B., *Am. J. Physiol.*, 1924, lxi, 371.

² Kossel, A., and Dakin, H. D., *Z. physiol. Chem.*, 1904, xli, 321.

³ Ringer, M., Experiments toward an ultimate determination of the site of urea formation. Thesis, C. C. N. Y., 1915.

⁴ Benson, Jr., O. O., The formation of urea in the animal organism, Master's Thesis, State University of Iowa, 1925.

lar results have been obtained by several workers. Hoagland and Mansfield,⁵ contrary to their expectations, obtained entirely negative results in muscle autolyses. Fosse and Rouchelman,⁶ employing the xanthidrol method, were able to demonstrate a six fold increase of urea in autolyzing liver tissue, a change which was entirely inhibited by boiling the liver. Fosse⁷ believes that the intermediate substance in urea formation is cyanic acid. McCance⁸ has endeavored to ascertain the optimum conditions for urea formation in autolyses. He reports urea formation not only in liver but also in spleen.

Data illustrating the results which were obtained in our earlier experiments are given in Table I. As will be noted there is no

TABLE I. Muscle and Liver Autolyses (1914).

Tissue employed	Time autolyzed at 38° C.	Urea N	Ammonia N
	days	mg. per 100 gm.	mg. per 100 gm.
Muscle Cat 17	0	21.4	13.8
	4	22.4	22.4
	12	21.4	25.0
	16	23.3	26.1
	20	23.6	27.5
	28	25.7	28.6
	56	22.4	36.8
Liver Cat 18	0	7.0	23.3
	8	31.1	43.5
	14	87.1	74.6
	21	128.1	155.5

evidence of any urea formation in the muscle autolyses, but in the liver autolyses concentration of the urea increased more than ten fold.

In addition to estimations of urea and ammonia nitrogen our recent analyses have included estimations of amino acid nitrogen as well. A few of our observations are given in Table II. These do little more than confirm our earlier experiments. The slight rise in amino acid nitrogen does not appear to have any special significance. It will be noted that in the liver autolyses there was a 300 mg. increase in urea nitrogen in about five days. In the mixed muscle-liver autolyses there was a considerable in-

⁵Hoagland, R., and Mansfield, C. M., *J. Biol. Chem.*, 1917, xxxi, 487.

⁶Fosse, R., and Rouchelman, N., *Comp. rend. Acad. Sci.*, 1921, clxxii, 771.

⁷Fosse, R., *Bull. Soc. chim. biol.*, 1920, ii, 4.

⁸McCance, R. A., *Biochem. J.*, 1924, xviii, 487; 1925, xix, 134.

TABLE II. Muscle and Liver Autolyses (1925).

Tissue employed	Time autolyzed at 38° C.	Urea N	Ammonia N	Amino acid N
		mg. per 100 gm. of tissue		
Rabbit muscle Exp. 7	hours			
	1	35.7	11.7	46.4
	12	34.8	18.6	46.6
	24	36.2	21.3	49.3
	48	34.3	22.1	57.2
	96	36.7	42.1	58.1
Calves' liver Exp. 10	2	46.7	27.7	46.0
	10	182.0	49.0	49.3
	20	319.5	55.2	50.5
	44	321.0	65.2	53.4
	88	325.0	67.1	55.5
	136	341.0	77.1	—
Mixture of rabbit muscle and liver 17 per cent liver Exp. 13	0	34.0	25.4	45.9
	12	37.6	35.8	49.4
	36	76.8	50.0	50.5
	84	94.9	60.1	54.4

crease in urea, but the amount was no greater than could be explained on the basis of the liver tissue alone.

There is no doubt that urea originates in the autolyses of liver tissue. Liver perfusion experiments have clearly shown that ammonium salts and some amino acids, but not all, give rise to urea.⁹ These substances are probably not the immediate precursors of urea and it would seem likely that there was an intermediate stage in urea formation. We believe that suitably planned autolyses experiments with liver tissue and extracts should disclose the immediate precursor of urea, although efforts in this direction have thus far been negative.

⁹ Löffler, W., *Biochem. Z.*, 1916, lxxvi, 55.

Action of cocaine on pupil compared with action on other
structures containing smooth muscle.

G. H. MILLER.

[*From the Department of Pharmacology, State University of
Iowa, Iowa City, Ia.*]

The mydriatic effect of cocaine is regarded by some to be due to a stimulation of the peripheral sympathetic nervous mechanism supplying the radial muscle of the iris. By others it is regarded as the result of a direct weakening of the circular muscle of the iris.

The first group of experiments here reported were performed in an attempt to demonstrate by actual measurements such a weakening of the circular muscle by cocaine. Accurate pupillary measurements were obtained by measuring the image of the pupil on a scale in the eye-piece of a measuring telescope. Series of measurements made in this way were employed for the plotting of curves showing the development of drug effects.

Albino rabbits were the animals employed. In the study of cocaine, two drops of a four per cent solution were placed in one eye only. After five to ten minutes there was placed in each eye a definite amount of solution of physostigmine sulphate. Frequent measurements were made of both pupils, and curves were plotted to show the effect exerted by the cocaine on either the degree of the physostigmine effect or on the rate of its development.

Thirty-six experiments were carried out, some on animals from which the superior cervical sympathetic ganglion on one side had been removed, and some on animals on which this operation had been performed on both sides. A few experiments were on unoperated animals. This work gave no evidence of a weakening of the circular muscle of the iris by cocaine.

The second group of experiments is a study of the effect of cocaine on the isolated sphincter muscle of the iris, mounted in Locke-Ringer solution for the recording of its muscular activity as a kymographic tracing. Steers and large dogs were used.

Cocaine in 1:10,000 concentration produced no effect. This failure to obtain any effect with cocaine is not due to a loss of

the activity of the muscle, for it still contracts in the usual manner after applying physostigmine, and relaxes after the use of adrenalin or atropine. Cocaine in concentrations of 1:1,000 or stronger caused some relaxation of the muscle. Such concentrations are, however, too high to represent the concentration of cocaine which reaches the iris in clinical ophthalmologic use. This is indicated by the observation that while 1:1,000 cocaine readily produces an anesthetic effect, anesthesia of the iris does not occur after the usual clinical instillation into the conjunctival sac. The severe pain felt by the patient when the iris is cut during an operation for cataract is an evidence of this.

The third group of experiments is a study of the effects of cocaine on isolated tissue from certain other structures containing smooth muscle having a sympathetic nerve supply. This was done in a search for evidence as to whether cocaine may stimulate the sympathetics in smooth muscle other than the iris. The uterus of the rabbit, both pregnant and non-pregnant, was always markedly stimulated by cocaine (1:10,000). Both rate of contractions and tone are increased. The pregnant guinea pig shows a similar effect. Tracings showing the action of cocaine in these experiments are almost duplicates of control tracings made at the same time, using adrenalin (1:1,000,000) on a similar piece of tissue.

The uterus of the non-pregnant cat, in which the sympathetics are inhibitory, responds to cocaine (1:10,000) with a complete and rather prolonged inhibition of contractions. There is a primary rise of tone followed by a prolonged, marked relaxation. Adrenalin controls showed a similar effect, but much shorter in duration and without the primary rise in tone.

Isolated segments of small intestine show an inhibition of contractions and a loss of tone after the use of cocaine. There is a following rise in tone during which the contractions remain inhibited as to amplitude.

The evidence of these experiments has all pointed towards a stimulation of sympathetics rather than to a weakening of smooth muscle by cocaine in dilating the pupil. There was found:

1. No measurable weakening of the circular muscle of the iris by cocaine in the intact animal.
2. No relaxation of the isolated sphincter of the iris by cocaine in 1:10,000 concentration.

3. Isolated smooth muscle of uterus and intestine gave evidence of stimulation by cocaine, 1:10,000, in tissues where the sympathetics are motor. Where the sympathetics are inhibitory, on the other hand, an inhibition of rhythmic contractions was always obtained following the use of 1:10,000 cocaine. The tonicity of the muscle in the latter case showed a phase of inhibition and a phase of increased tone.

3023

Comparative metabolism of hydantoin and beta-methyl
hydantoin.

O. H. GAEBLER.

[*From the Biochemical Laboratory, State University of Iowa,
Iowa City, Iowa.*]

In previous experiments¹ it was pointed out that when beta-methyl hydantoin is given subcutaneously to dogs only fifteen per cent of the compound reappears unchanged in the urine. A similar amount is excreted as methyl hydantoic acid. But while all of the nitrogen of the compound was excreted, the remainder was unaccounted for in the nitrogen partition. Immense amounts of oxalic acid were found in the urines during experimental days, and this suggested hydrolysis of methyl hydantoic acid to methyl urea and glycollic acid, with oxidation of the latter to oxalic acid. Supplementary experiments have thrown doubt upon excretion of a large amount of methyl urea, hence the possibility of origin of the oxalic acid found from a substance such as methyl parabanic acid, in the method of determination, is being considered.

Experiments have also been carried out in which hydantoin and hydantoic acid were administered. In keeping with the findings of Lewis² no evidence of urea or ammonia formation was obtained. When hydantoin was given there was, however, an increase in organic acid excretion corresponding to elimination,

¹ Gaebler, O. H., *Proc. Am. Soc. Biol. Chem.*, 1925, lvii, 55.

² Lewis, H. B., *J. Biol. Chem.*, 1912-13, xiii, 347.

as hydantoic acid, of one-half of the compound administered. Similarly, the apparent rise in creatinine excretion, due to elimination of unchanged hydantoin, was only half as great as it would have been had all of the compound been excreted unchanged. The rise in total nitrogen again accounted for all of the compound, and the amount of benzal hydantoin,³ isolated from the urine after hydrolysis, justified the assumption that all of the compound is excreted either unchanged or as the corresponding acid.

While hydantoin therefore undergoes partial hydrolysis to the corresponding hydantoic acid, it is very resistant to further destruction in the body, as compared with the previously investigated methyl derivative.

³ Wheeler, H. L., and Hoffman, C., *Am. Chem. J.*, 1911, xlv, 368.

Southern Branch

Tulane University, February 10, 1926.

3024

Fermentation phenomena when different species of micro-organisms are in close association.

ALDO CASTELLANI.

[*From the Department of Tropical Medicine, Tulane University, New Orleans, La.*]

During the years 1904 and 1905 I became interested in the study of baker's yeast and in fermentation caused by yeast. I made the following observations, mentioned at meetings of the Ceylon Branch of the British Medical Association:—

(1) Baker's yeast both in the East and in London is not a pure culture of a single species of yeast. It is generally composed of two or more species of yeast plus one or two or several species of bacteria. The bacteria isolated from these samples of yeast do not produce gas fermentation in sugars; they produce simple acidity (lactic acid fermentation).

(2) Comparing the fermentation reaction of baker's yeast used *in toto* as obtained from the bakers, sowing particles of the sample in the various sugars, with the fermentation reactions of the isolated pure cultures of the different species of saccharomyces and bacteria composing the sample, I found that the yeast *in toto* ferments a much larger number of carbohydrates than any one of the saccharomyces and bacteria composing the sample. In this country I may say that all the samples of yeast I have examined contain one or more gas forming bacilli.

(3) By mixing artificially all the organisms isolated from the sample of yeast in certain cases the same fermentation reactions are obtained as with the yeast *in toto*. There are apparent exceptions as shown by the following example:

From Orphington, a place in the country near London, I obtained in 1919 a sample of baker's yeast which gas fermented a very large number of carbon compounds, including starch. I in-

vestigated the sample mycologically by plating on maltose and glucose agar, etc., and I isolated and grew in pure cultures what I believed to be all the organisms, saccharomyces and bacteria found in that sample. By mixing all the pure cultures I obtained the same fermentation reactions as with the sample of yeast *in toto* except that the mixture did not touch starch. The experiment was repeated many times with the same result, until finally I had recourse to the following procedure: The surface of the glucose agar plates on which a minute particle of the yeast cake had been plated out were washed with a little peptone water and the washing inoculated into starch. Fermentation took place, the inference being that probably some very minute colonies on the verge of invisibility had escaped my notice when investigating the plates and picking out all the colonies present; the thought came to me also that there might be an ultramicroscopic germ present.

(4) By mixing artificially a non-maltose fermenting saccharomyces (isolated from a sample of baker's yeast) with a bacillus, isolated from the same sample, producing acidity in maltose and several other sugars but never gas in any substance, I obtained fermentation of maltose with production of gas.

(5) By mixing artificially two species of saccharomyces, neither of which gas-fermented saccharose, gas-fermentation of this sugar was obtained.

In recent years I have studied the same and similar phenomena, using mostly pathogenic bacteria: bacilli and cocci, also a pathogenic yeast. In the present paper I will limit myself to give the results of experiments, a number of them already published, with the following organisms:

(a) *Bacilli*: *B. typhosus*, *B. dysenteriae Flexner*, *B. morgani*, *B. proteus*, strain P., *B. coli communior*, *B. kandiensis*, *B. vermiculoides*.

(b) *Cocci*: *Staphylococcus aureus*, two strains of *Streptococcus pyogenes*.

(c) *Yeast*: *Cryptococcus graciloides*.

B. TYPHOSUS + B. MORGANI

Action on Maltose. *B. typhosus* alone produces acidity, never gas. *B. morgani* alone has no action whatever on maltose; it produces neither acidity nor gas. If a tube of maltose peptone water is inoculated, however, with *B. typhosus* and immediately after with *B. morgani* and placed in the incubator at 35° C. or

37° C. for 48 hours, not only will acidity be produced, but also a large amount of gas. The same result is obtained if the two bacilli are previously mixed in plain peptone water and the peptone water mixture inoculated in tubes of maltose. The same result is also obtained if *B. morgani* is added to *B. typhosus* 24 hours after inoculating with *B. typhosus*; if the interval is more than 24 hours then the production of gas is not so constant.

When the two bacilli, *B. typhosus* and *B. morgani*, live naturally in close association the same phenomenon takes place as shown by the following observation, which I related in one of my previous papers. The stool of a suspected typhoid case was examined bacteriologically in London, using McConkey's medium plates. An exceptionally large number of white colonies developed; fifteen were picked out and further investigated by replating, etc. Twelve of them consisted of two organisms—*B. typhosus* and *B. morgani*; three of *B. morgani* alone. The mixed cultures derived from the colonies containing only *B. morgani* had no action whatever on that sugar. Mixed cultures obtained artificially by inoculating agar tubes with both *B. typhosus* and *B. morgani* behave in the same manner—namely, when a loopful of the mixed growth is inoculated into a maltose peptone water tube, acid and gas are produced.

Action on Mannitol. As is well known *B. typhosus* produces acidity only in mannitol, never gas; *B. morgani* has no action—no acidity or gas is produced. The mixture *B. typhosus* + *B. morgani* produces acidity and gas.

Action on Sorbite. *B. typhosus* alone produces acidity, never gas, in sorbite; *B. morgani* alone has no action whatever on sorbite; it produces neither acidity nor gas. The mixture *B. typhosus* + *B. morgani* produces acidity and gas.

TABLE I.

	Lactose	Saccharose	Glucose	Levulose	Maltose	Galactose	Mannitol	Sorbite
<i>B. typhosus</i>	0	0	A	A	A	A	A	A
<i>B. morgani</i>	0	0	AG	AG	0	AG	0	0
<i>B. typhosus</i> + <i>B. morgani</i>	0	0	AG	AG	AG	AG	AG	AG

O = Negative, neither acid nor gas. A = Acid. G = Gas.

Action on other Carbon Compounds apart from the above. The reactions are seen at a glance in Table I, in which the fermentative characters are given of *B. typhosus* alone, *B. morgani* alone, and the mixture of *B. typhosus* + *B. morgani*.

From the table it is seen that certain sugars and other carbon compounds, which when acted upon by *B. typhosus* alone undergo a simple acid fermentation, and when acted upon by *B. morgani* alone do not undergo any fermentation, neither acid nor gas being produced, are capable of undergoing fermentation with production of gas when they are acted upon by the mixture of the two bacilli. In the carbon compounds in which *B. typhosus* alone produces no acidity and *B. morgani* alone neither acidity nor gas their mixture produces neither acidity nor gas.

B. TYPHOSUS + B. PROTEUS

The strain of *B. proteus* used (P), as already stated, has no action on maltose, mannitol, or sorbite; it produces neither acidity nor gas in these substances.

Action on Maltose. *B. typhosus* alone produces acidity only, never gas; *B. proteus* (P) alone produces neither acidity nor gas; their mixture produces acidity and gas.

Action on Mannitol. *B. typhosus* produces acidity, never gas, in this alcohol. *B. proteus* (P) has no action on it; neither acidity nor gas is produced. Their mixture, however, produces acidity and gas.

TABLE II.

	Lactose	Saccharose	Glucose	Levulose	Maltose	Galactose	Mannitol	Sorbite
<i>B. typhosus</i>	0	0	A	A	A	A	A	A
<i>B. proteus</i> (P)	0	0	AG	AG	0	AG	0	0
<i>B. typhosus</i> + <i>B. proteus</i> (P)	0	0	AG	AG	AG	AG	AG	AG

0 = Negative, neither acid nor gas. A = Acid. G = Gas.

Action on Sorbite. *B. typhosus* produces acidity only; *B. proteus* neither acidity nor gas; their mixture causes production of acidity and gas.

B. DYSENTERIAE FLEXNER + B. MORGANI

Action on Mannitol. *B. dysenteriae Flexner* produces acidity only, never gas; *B. morgani* has no action on it; neither acidity

nor gas is produced. The mixture *B. dysenteriae* + *B. morgani* produces acidity and gas.

Action on Maltose. *B. dysenteriae Flexner* produces only acidity, never gas; *B. morgani* produces neither acidity nor gas; their mixture produces acidity and gas.

B. DYSENTERIAE FLEXNER + B. PROTEUS

Action on Mannitol. *B. dysenteriae Flexner* produces acidity, never gas; *B. proteus* (P) produces neither acidity nor gas; their mixture produces acidity and gas.

Action on Maltose. *B. dysenteriae Flexner* produces only acidity, never gas; *B. proteus* (P) produces neither acidity nor gas; their mixture produces acidity and gas.

TABLE III.

	Lactose	Saccharose	Glucose	Levulose	Maltose	Galactose	Mannitol	Sorbite
<i>B. dysenteriae Flexner</i>	0	0	A	A	A	A	A	0
<i>B. morgani</i>	0	0	AG	AG	0	AG	0	0
<i>B. proteus</i> (P)	0	0	AG	AG	0	AG	0	0
<i>B. dysenteriae Flexner</i> + <i>B. morgani</i>	0	0	AG	AG	AG	AG	AG	0
<i>B. dysenteriae Flexner</i> + <i>B. proteus</i> (P)	0	0	AG	AG	AG	AG	AG	0

O = Negative, neither acid nor gas. A = Acid. G = Gas.

B. TYPHOSUS + B. MORGANI + B. PROTEUS

Action on Maltose, Mannitol and Sorbite. *B. typhosus* alone produces acidity, no gas. *B. morgani* alone produces neither acidity nor gas. *B. proteus* (P) alone produces neither acidity nor gas. The mixture *B. morgani* + *B. proteus* produces neither acidity nor gas. The mixture of the three germs produces acidity and gas.

B. COLI COMMUNIOR + B. KANDIENSIS (VAR. KANDIENSOIDES)

Action on Adonite. *B. coli communior* produces neither acidity nor gas. *B. kandiensis*, acidity only, never gas. The mixture *B. coli communior* + *B. kandiensis* will produce acidity and gas, although slowly. The tubes must be kept at 37° C. for at least a week.

STAPHYLOCOCCUS AUREUS + B. MORGANI

Action on Maltose. *Staphylococcus aureus* alone (Ross Institute Strain) produces acidity, no gas in maltose; *B. morgani*

alone produces neither acidity nor gas. The mixture *Staphylococcus aureus* + *B. morgani* produces acidity and gas.

STAPHYLOCOCCUS AUREUS + B. PROTEUS

Action on Maltose. *Staphylococcus aureus* alone (Ross Institute Strain) produces acidity, no gas in maltose; *B. morgani* alone produces neither acidity nor gas. The mixture *Staphylococcus aureus* + *B. proteus* produces acidity and gas.

STREPTOCOCCUS PYOGENES + B. MORGANI

Action on Maltose. *Streptococcus pyogenes* (Strain Public Health Laboratory, London,) alone produces acidity, no gas; *B. morgani* produces neither acidity nor gas. The mixture *Streptococcus pyogenes* + *B. morgani* produces acidity and gas, but it may take eight or ten days at 37° C. before the gas appears.

STREPTOCOCCUS PYOGENES + B. PROTEUS

Action in Maltose. *Streptococcus pyogenes* (Strain Public Health Laboratory, London,) alone produces acidity, no gas; *B. proteus* produces neither acidity nor gas. The mixture *Streptococcus pyogenes* + *B. proteus* produces acidity and gas, but it may take eight or ten days at 37° C. before the gas appears.

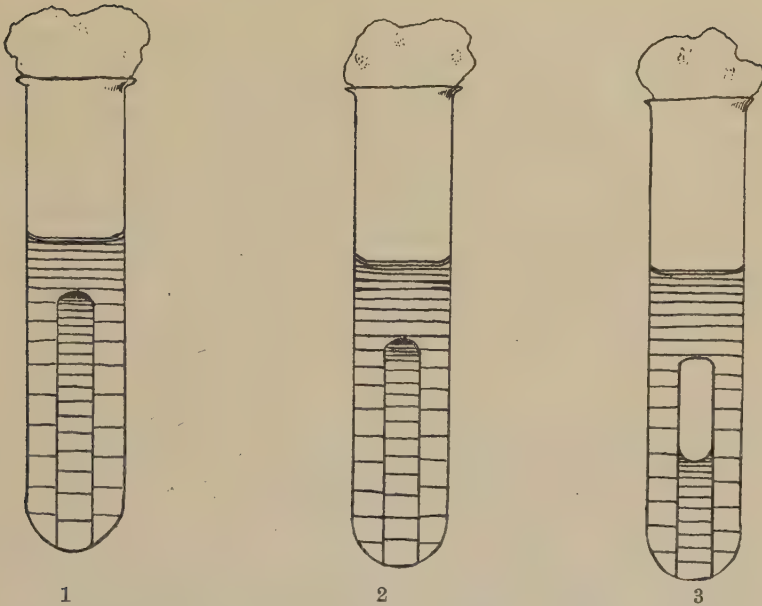
BACILLUS VERMICULOIDES + CRYPTOCOCCUS GRACILOIDES

These two organisms live apparently in symbiosis and it took me more than a year to separate them. The symbiotic mixture produces, though very slowly, gas in glucose, levulose and maltose. The cryptococcus alone does not produce either acid or gas; the bacillus alone produces acidity only.

TABLE IV.

	Lactose	Glucose	Levulose	Maltose	Galactose	Saccharose	Mannite	Dulcite	Salicin	Inosite	Dextrin	Inulin	Sorbite	Adonite	Arabinose	Raffinose	Insodulcite	Erythrite	Glycerine	Gelatine
<i>Cryptococcus graciloides</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Castellani</i>																				
<i>Bacillus vermiculoides</i>	A	A	A	A	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Castellani</i>													0 or A v. S							
<i>Cryptococcus graciloides</i>																				
+																				
<i>B. vermiculoides</i>	A	AG	AG	AG	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

A = acid. G = Gas. O = absence of gas or coagulation. S = slight.



1. Manitol peptone water tube inoculated with *B. dysenteriae Flexner*; gas absent. 2. Mannitol peptone water tube inoculated with *B. morgani*; gas absent. 3. Mannitol peptone water tube inoculated with *B. dysenteriae Flexner* + *B. morgani*; gas present.

RESUME AND CONCLUSIONS

"Baker's yeast" as obtainable in Ceylon and in England is not a pure culture of yeast, but is generally composed of two or more species of *Saccharomyces* and one or two or several species of bacteria living in close association. The whole yeast as a rule gas-fermented more carbohydrates than any of the isolated germs did, either *Saccharomyces* or bacteria. A bacillus, which produced only acidity, not gas, in maltose, would produce gas when *Saccharomyces* was added to it, was inactive on maltose and was isolated from the same sample.

In recent years I have used for my experiments mostly pathogenic bacteria and also a pathogenic yeast, *Cryptococcus graciloides*.

Simple acid fermentation of certain carbon compounds caused by micro-organisms which never produce gas may in a number of instances be transformed into fermentation with the production of gas by adding a micro-organism which, alone, is inert, viz., produces neither acidity nor gas on those particular carbon

compounds. For example: *B. dysenteriae Flexner* produces acidity, never gas, in maltose and mannitol; *B. morgani* produces neither acidity nor gas in those two carbohydrates; the mixture *B. dysenteriae Flexner* + *B. morgani* produces, however, acidity and gas. For the phenomenon to take place several conditions are necessary; the most important one seems to be that the added bacillus, though inert on those particular carbon compounds, must be capable of producing fermentation with gas in glucose.

3025

Electrocardiographic studies of the action of propylene and some other anesthetic gases.

A. M. CAINE and CHAPMAN REYNOLDS. (Introduced by J. T. Halsey).

[From the Department of Pharmacology and Therapeutics,
School of Medicine, Tulane University, New Orleans, La.]

Earlier experimental observations¹ and certain clinical experiences indicated the advisability of making an electrocardiographic study of the behavior of the heart under the influence of propylene, the results of which are here reported.

In four dogs and three cats the administration of propylene in concentrations from 25 per cent (less than sufficient to induce or maintain anesthesia) up to 40-50 per cent caused ectopic* ventricular beats, which promptly ceased when the gas was discontinued or the concentration lowered. With higher concentrations the ectopic beats usually became more and more numerous, often so much so that an ectopic ventricular tachycardia resulted. Under these conditions also discontinuance of the gas or a sufficient lessening of its concentration resulted in a return to the normal mechanism. In control observations with ether, chloroform, nitrous oxide, ethylene, and acetylene in varying concentrations ectopics were not observed.

¹ Halsey, J. T., Reynolds, C., and Prout, W. A., *J. Pharm. and Exp. Ther.*, 1925, xxvi, 479.

* Originating at various points in each ventricle.

3026

Blood platelets in rabbits following splenectomy and transplantation of the spleen.*

R. T. LILES. (Introduced by Isadore Cohn).

[*From the Department of Medicine, Tulane University, La.*]

While cooperating with Drs. Isidore Cohn and I. I. Lemann during the course of some experimental work on rabbits, observations were made on the blood platelets following splenectomy and transplantation of the spleen.

Under ether anaesthesia the spleen was removed and the whole organ immediately transplanted beneath the rectus sheath of another etherized animal. After a period of 25 to 35 days the animals were killed. At post-mortem the grafts were found to have been completely destroyed and largely replaced by a necrotic mass, all transplants being considered non-functional.

Blood platelet counts were made before operation and at intervals of 24 to 48 hours after operation until the animal was killed, covering the period of 25 to 35 days. The average number of platelets in all animals before operation was found to be 375,000 per cm. of blood. Forty-eight hours after operation there was an average of 475,000 per cmm. in the splenectomized group, and an average of 573,000 per cmm. in the group of recipients. During the first ten days after operation this primary increase was sustained, the number of platelets being approximately the same in both groups on the tenth day, averaging 476,000 per cmm. in the donor group and 466,000 per cmm. in the group of recipients. After the tenth day the number of platelets gradually decreased in the recipient group, reaching the preoperative level about the twentieth day and remaining normal thereafter. In the other group the increase was sustained throughout the entire period of postoperative observation. Two animals of this group were observed over a period of 35 days after operation, at the end of which there was approximately 500,000 platelets per cmm. present in each case, or approximately 125,000 per cmm. more than before operation.

The initial increase in platelets in both groups of cases we at-

* Work done under Swartz Research Fund, Tulane University, La.

tribute to the response of the organism to traumatism, the sustained increase in the group of donors we attribute to the absence of the destructive properties of the spleen for blood platelets.

Technique employed in counting platelets: considerable difficulty was experienced in finding a method by which we were able to get accurate results. A large number of methods, both direct and indirect, were tried. The chief difficulty encountered in making direct count was in obtaining a satisfactory diluting fluid. Various anti-coagulants were used with and without the addition of dyes. Of these solutions those without the addition of dye were far more satisfactory. The difficulty with the clear fluids was the ever present tendency of the platelets to clump together, while with the solutions containing the dye there was added the great difficulty of differentiating the platelets from certain bacteriae and other foreign bodies that take a similar stain. Of the indirect methods the technique described by J. H. Pratt¹ proved most satisfactory, but because of the time consumed in making these counts all indirect methods were considered impracticable. However, while using Pratt's technique it was observed that clumping rarely occurred in the preparation. Hence, the idea was evolved to use two per cent sodium metaphosphate made up in normal saline as a diluting fluid in making direct counts.

In the direct method the platelets are counted as in the enumeration of the red blood corpuscles, except that the following points must be observed:—

1. The puncture must be sufficient to obtain the flow of blood immediately with minimum manipulation.
2. The suspension must be promptly and thoroughly mixed.
3. The mount should be allowed to stand from 8 to 10 minutes before making the count.
4. At least twice as many fields should be counted as is done in enumerating the red blood corpuscles.

In our hands this method has been very satisfactory. The tendency of the platelets to clump is practically overcome and we have been able to check counts accurately.

¹ Osler, Wm., and McCrae, *Modern Medicine*, Vol. iv, 694.

3027

The fate of xylose in the animal body.

RALPH C. CORLEY. (Introduced by W. Denis).

[From the Department of Bio-Chemistry, College of Medicine,
Tulane University, New Orleans, La.]

The very similar chemical properties, correlated with closely corresponding structural configurations of the pentoses and hexoses, make difficult the elaboration of chemical methods for the determination of one in the presence of the other. The resistance of pentoses to fermentation by ordinary baker's yeast has furnished a means that has been used at times to separate the two types of substances for analytical purposes. In the present study this procedure has been employed to determine the pentoses in the blood following their administration intravenously. The reducing power of normal blood can not be removed completely by fermentation with yeast, but since this residual reduction seems to remain small and fairly constant no real difficulty arises. It is scarcely unjustifiable to assume that in the presence of pentoses the difference from the usual unfermented reducing power of the blood furnishes a reliable index of the amount of pentoses present.

The details of this series of experiments may be summarized as follows: One gram of xylose in 15 cc. of solution was injected into the marginal ear vein of a 2 to 2.4 kg. fasting rabbit. The blood samples (2 to 3 cc.) were collected at the following intervals: one the preceding day, one just before and one just after the experiment, others after one, two, four and eight hours, respectively. These were analyzed for non-protein nitrogen, blood sugar and reducing power after fermentation. For this latter determination the whole blood was incubated one hour at 37 C. with a suspension of yeast. Then the proteins were precipitated according to the Folin-Wu procedure, and the reducing power of the filtrate determined by the Shaffer-Hartmann method, the results being expressed in terms of glucose.

In general, the total blood sugar paralleled the unfermented fraction. In the control experiments there was no change in the residual reduction in the normal rabbits or those with phlorhizin diabetes. Tartrate nephritis is associated with a pronounced in-

crease and chloroform poisoning with a less marked increase in the residual reduction, the changes being accompanied by a rise in the non-protein nitrogen, to which they are undoubtedly due in large measure. After the injection of one gram of glucose, while there was the usual marked temporary hyperglycemia, there was no change in the unfermented residuum. Subsequent to the administration of xylose the reducing power of the blood after fermentation returned to its previous value in four hours in the normal rabbit, two hours in phlorhizin diabetes, four hours in chloroform poisoning, but was scarcely back to the control level in eight hours in tartrate nephritis.

3028

Evidence for the supernormal phase and a recovery curve of conduction in the human heart.

RICHARD ASHMAN and GEORGE R. HERRMANN.

[*From the Departments of Physiology and Medicine, Tulane University, New Orleans, La.*]

Recent work by Ashman¹ and by Ashman and Woolley² has demonstrated that the compressed or injured cardiac muscle of the turtle may, after excitation, recover its conductivity along a curve which passes through a supernormal phase. Thus an impulse traversing the compressed muscle will travel most rapidly during the supernormal period. If the degree of compression is sufficient only impulses arriving at the compressed region during the supernormal phase are transmitted; earlier or later ones are blocked. These experimental observations add much weight to the argument for a supernormal phase in the recovery of conductivity in the human hearts discussed below.

Lewis and Master³ reported two cases of block in the human heart which they interpreted as evidencing a supernormal phase. In their first case typical complete heart block was interrupted at

¹ Ashman, Richard, *Am. J. Physiol.*, 1925, lxxiv, 140.

² Ashman, R., and Woolley, E., *Proc. Soc. Exp. Biol. and Med.*, 1925, xxiii, 159.

³ Lewis, T., and Master, A. M., *Heart*, 1924-25, xi, 371.

times by a ventricular systole in response to an auricular impulse and only when the latter arose during late systole or early diastole. Auricular impulses arising later in diastole were blocked.

We have obtained electrocardiograms of two clinical cases of heart block which show phenomena attributable to a supernormal phase. Our first case is similar to Lewis and Master's excepting that, (a) a series of auricular impulses are transmitted if the auricles are rapid; (b) ventricular standstill supervenes when, as a result of auricular slowing, conduction fails, and (c) the period of ventricular quiescence is interrupted at irregular intervals by idioventricular contractions which do or do not permit conduction depending upon their time relations with the auricular systoles. As in Lewis and Master's case, conduction time increases slightly as the interval between the R-wave and the subsequent P-wave widens.

In our second case most of the P-waves which fall between about 0.06 sec. before the R and 0.36 sec. after the R-wave are transmitted. Earlier and later impulses are blocked. There is some overlapping of blocked and transmitted impulses at either end of this range.

The conduction times (P-Q) vary from 0.60 to 1.01 second. Their average duration exceeds the average length of the auricular cycles. Unlike Lewis and Master's first case and our first case, the conduction times become progressively shorter with longer ventricular rest until block suddenly supervenes. These facts may be explained on the assumption that only a very short stretch of the conducting tissue recovers through a supernormal phase, and that the greatly prolonged conduction times are due to time occupied by the impulse in passing other damaged tissue.

When corrections are made for variations in the auricular rate and for the duration of the rest period on the ventricular side of the block,⁴ a curve of recovery of conductivity is obtained of a form similar to those determined in animal experiments.^{5, 6} The curve demonstrates that in the depressed conducting tissue of this heart conductivity had not fully recovered at the end of more than a second.

⁴ Herrmann, G. R., and Ashman, R., *Am. Heart J.*, 1926, i, 269.

⁵ Ashman, Richard, *Am. J. Physiol.*, 1925, lxxiv, 121.

⁶ Lewis, T., and Master, A. M., *Heart*, 1925, xii, 209.

Minnesota Branch

University of Minnesota, February 3, 1926.

3029

Iodine and goiter in Utah and use of the Cottrell precipitator in iodine analysis.

J. F. McCLENDON.

[From the Laboratory of Physiological Chemistry, University of Minnesota, Minneapolis, Minn.]

In reviewing the iodine determinations of drinking water from the State of Utah made in this laboratory (some of these determinations being made by J. C. Hathaway, H. H. Vanderslins, Clarence Dahl) it was found there was not a very close relationship between iodine and freedom from goiter. In some cases a number of determinations were made on the same water supply and the iodine content was found to vary at different times.

In the case of the water supply of Stanford University a chemist who had worked on these iodine determinations actually collected and evaporated the water and yet it varied considerably during different months.

Since the enlargement of the thyroid is the result of iodine deficiency during several years, even though the child has not changed its residence during this period, the immediate iodine content of the water supply would not be expected to bear a very close relation to the size of the thyroid. If, however, a large number of cases are taken it is always found that where there is a low average iodine content of the water there is higher average incidence of goiter among school children as shown in the following table:

TABLE I.

Iodine in parts per billion	City	County	Per cent goiter in schools
.01	Ogden	Weber	53.
	Parowan	Iron	69.1
	Vernal	Uintah	40.8
	Nephi	Juab	64.3
	Kamas	Summit	46.4
	Park City		42.2
	Salt Lake City	Salt Lake	41.6
.16	Alpine		57.
.33	Syracuse	Davis	23.1
	Millcreek	Salt Lake	42.5
.36	Oak City	Millard	82.2
.4	Logan City	Cache	40.5
	Ft. Duchesne	Ninta	71.7
			Average 52
.5	Murray	Salt Lake	34.4
	W. Warren		7.1
.6	Brigham City	Boxelder	29.0
	Santaquin		45.
	Butler School	Salt Lake	67.3
.7	Farmington	Davis	54.5
	Huntsville	Weber	41.1
.6	Kaysville	Davis	49.1
.9	Mt. Pleasant	Sampete	58.6
	Levan	Juab	73.4
2.16	Lakeview		6.
2.5	Goshen		15.
9.5	Milford	Beaver	23.
			Average 39

In this table there are twenty-six towns. Thirteen with low iodine show an average of 52 per cent goiter in the schools. The thirteen with high iodine show an average of 39 per cent goiter in the schools. One town, Huntsville, in Weber County, with 41.1 per cent goiter in the school, showed on one analysis high iodine (7.07), and in another analysis low iodine (0.22). It was included in the table, and yet its inclusion in either the high or the low would not materially affect the average. At Kaysville one analysis showed 0.06 iodine and a later one 1.6. Both of these would be in the high group. The figure for Alpine is for Alpine Canyon, Grove Creek having 0.43 I₂. In order to get a closer correlation it is necessary to determine the iodine in the foodstuffs since usually the pupil gets more iodine from foodstuffs than water and the foodstuffs are not always grown on the water-shed from which the water comes.

In the determination of iodine in foodstuffs it is necessary to completely burn the organic matter and it is difficult to collect all

of the iodine from the smoke. The smoke may be passed through a number of wash bottles and not catch all the solid particles. The method of passing it between a hot and cold tube is somewhat cumbersome. The Cottrell precipitator described by Harold C. Webber¹ was found to be effective provided that the smoke did not pass through it too rapidly. The apparatus was simplified in that the beaker was dispensed with, the side arms put on at right angles and a platinum wire was fastened in the center of the precipitated tube by passing it through rubber stoppers at the two ends of the tube and attaching it to one of the secondary binding posts of the Ford spark coil. A split brass tube was clamped around the outside of the lead glass precipitating tube and attached to the other secondary terminal of the coil (the common or ground terminal). In burning a pound or more of foodstuff it is necessary to have a battery of these precipitators or make the tube much longer. The smoke sticks to the platinum wires and they are withdrawn and coiled around a silica rod and burned in a small combustion tube to free the iodine.

3030

Composition of rats on low magnesium diet.

GRACE MEDES. (Introduced by J. F. McClendon).

[*From the Laboratory of Physiological Chemistry, University of Minnesota, Minneapolis, Minn.*]

Three rats were fed on diets of purified casein, glucose (cerealose) and cod liver oil, with a salt mixture of NaCl , CaCO_3 , K_2HPO_4 and $\text{Fe}_2(\text{SO}_4)_3$. Analysis of the ash of samples of these diets showed that they contained 1.2 mg. magnesium per 100 gm. of diet. The rats were kept on the diets 20, 20 and 100 days respectively. The ash of the rats contained 23, 14 and 31 mg. magnesium, or 0.030, 0.029 and 0.026 per cent of their respective body weights. The corresponding control rats contained 24, 15 and 36 mg., which was .039, .043 and .037 per cent

¹ Webber, H. C., *J. Ind. and Eng. Chem.*, 1924, xvi, 1241.

of their respective body weights. Estimating that rat No. 3, which weighed 52 gm. at the beginning of the experiment, contained about .040 per cent magnesium, or 21 mg. magnesium, it gained approximately 10 mg. During the same period, it ate about 1000 gm. diet, containing 12 mg. It must, therefore, have absorbed and retained nearly all the magnesium it received. This is in marked contrast to its inability to utilize all of the Ca and P of a diet, even when they are present in inadequate amounts.

3031

A report on the preparation of pneumococcic antitoxin.**W. P. LARSON.**

*[From the Department of Bacteriology and Immunology,
University of Minnesota, Minneapolis, Minn.]*

In the present paper I wish to make a brief report of results obtained in an effort to produce a pneumococcic antitoxin. This work is the outgrowth of our observation that bacterial toxins are rendered non-toxic by soaps of some of the unsaturated fatty acids, particularly sodium ricinoleate, and that the antigenic properties of such detoxified toxins remain unimpaired. We have further called attention to the fact that pneumococci, streptococci and certain other micro-organisms are either killed in a short time in aqueous solutions of sodium ricinoleate, or lose their powers to infect. Larson and Nelson¹ showed further that there is a rapid development of agglutinins in rabbits against both pneumococci and streptococci following the injection of soaped cultures of these organisms.

Ten cc. or more of a virulent broth culture of soaped pneumococci may be injected intraperitoneally into rabbits without causing an infection. Agglutinins appear in the blood stream as early as 24 hours following such injections. This observation led us to attempt to produce an antipneumococcic serum by this method. Pneumococci of various types were passed through a series of mice in order to increase their virulence as much as pos-

¹ Larson, W. P., and Nelson, E., *PROC. SOC. EXP. BIOL. AND MED.*, 1925, **xxii**, 357.

sible. They were then grown in veal infusion broth which contained 1 to 2 per cent peptone. Parke-Davis' and Witte's peptones were used, since these products have been found by others to be best for toxin production. The whole cultures were treated with sodium ricinoleate solutions of a pH of 7.8, so the final concentration of the soap was 1 per cent. The pH of the cultures was adjusted to 7.4 before the soap was added.

Rabbits and sheep were then immunized with these soaped cultures. Sheep were given as much as 200 cc. at one time. It was found that the blood serum of immunized animals would cure infected rabbits in a large percentage of cases.

The serum was then tried out on cases of lobar pneumonia at the Minneapolis General Hospital during the late winter and spring of 1925. A preliminary report of our results has been published by Larson and Fahr.² The rapid drop in temperature following the administration of the serum and the relief of subjective symptoms indicated that the serum actually contained antitoxin.

Using our cultures, Olson³ succeeded in isolating a toxin which gave a skin reaction in rabbits and produced changes, such as hemorrhage and even hepatization, in the lungs of mice following intraperitoneal injection. Olson showed further that the toxin was neutralized by our serum, proving that it actually contains pneumococcic antitoxin. The writer found that the toxin isolated by Olson gave skin reactions in approximately 50 per cent of normal individuals, while three cases who had received antitoxin gave a negative test. We are thus able to standardize the antitoxin in terms of the number of skin doses of toxin which are neutralized by one cc. of serum. Our antitoxin is now being used experimentally in several medical centers.

² Larson, W. P., and Fahr, *Minnesota Medicine*, 1925, viii, 424.

³ Olson, J. G., *Proc. Soc. Exp. Biol. and Med.*, 1926, xxiii 295-334.

3032

The water depots of the body.**H. P. SKELTON.** (Introduced by F. H. Scott).

[*From the Department of Physiology, University of Minnesota, Minneapolis, Minn.*]

It is well known that hemorrhage causes fluid to enter the blood from the tissues. Likewise it is well known that colloid-free fluids on injection rapidly leave the blood and pass to the tissues. It was the object of this study to determine, if possible, whether certain tissues are of more importance than others in these interchanges. The experiments were carried out on cats anesthetized with ether. The tissues chiefly worked with were skin (clipped free from hair), muscle, intestine, spleen, liver and blood. Both kidneys were ligated off. Corresponding parts on each side of the body were prepared and when all was ready, the small pieces from one side were snipped off and dropped into stoppered weighing bottles. Then the experimental procedure was performed and after thirty minutes the corresponding pieces from the opposite side of the body were taken. The samples were all dried to a constant weight at 102°. The experimental procedure consisted in a hemorrhage of 15 cc. per kilo or in the injection of 25 cc. of the various solutions per kilo. The calculations as to the amounts of the various tissues in the body of the cat were based on the figures of Voit.¹ The amount of blood in the cat was taken as 4.6 per cent of its weight and the amount of water entering or leaving the blood calculated from hemoglobin and hematocrit alterations.

The results are as follows: Following the hemorrhage all tissues examined gave up water, but of the amount of water entering the blood, about 78 per cent came from skin and subcutaneous tissue, 10 per cent from muscle, 8 per cent from the liver, 2 per cent from the intestine and .03 per cent from the spleen. The foregoing are the average of twenty-three experiments. The effect of intravenous injections may be seen from the following table:

¹ Voit, Carl von, *Z. f. Biol.*, II, 1886.

TABLE I.

Fluid injected 25 cc. per kilo	Per cent injected fluid leaving blood in 30 min.	Percentage of fluid leaving blood entering				
		Muscle	Skin	Liver	Intestine	Spleen
*1. Distilled water	97.8	36.0	8.0	12.0	6.0	0.25
2. 0.45% NaCl	96.0	40.0	7.6	23.0	9.6	0.40
3. 0.6% CaCl ₂	93.7	37.8	12.0	25.2	13.2	0.30
4. 0.9% NaCl	58.0	0.0	30.0	19.0	8.0	0.50
5. 1.8% NaCl	46.0	0.0	27.0	Samples lost	10.0	0.20

*1 is average of 10 experiments; 2, of 5; 3, of 5; 4, of 8; 5, of 6.

The table shows that with hypotonic solutions the fluid passes into all tissues; with isotonic solutions, however, little seems to enter muscle. In a few experiments not recorded in the table, stronger solutions of CaCl₂ were injected. It was found the fluid went chiefly into the intestine and the skin, the muscle actually losing fluid.

3033

Storage of water in various parts of the earthworm at different stages of exsiccation.

CLARENCE M. JACKSON.

[From the Department of Anatomy, University of Minnesota, Minneapolis, Minn.]

This study was made last December on 12 specimens of *Lumbricus terrestris*, recently obtained from Pennsylvania. When brought into the laboratory, the earthworms were kept 1 or 2 days barely covered with tap water until weight equilibrium was reached. The laboratory temperature during the experiment was nearly constant at 18° to 19° C., the extreme range being 16° to 20°.

Changes in body weight. Eight worms were exposed on dry filter paper, and weighed at frequent intervals. At various stages of exsiccation, they were replaced in tap water, to determine the rate of recovery in body weight. Two of the worms, after losing

42 or 43 per cent of their original weight in 4 or 5 hours, respectively, revived and nearly regained their original weight within 24 hours after replacement in water. The other 6 worms, in which the loss was 50 to 60 per cent after 5 to 9 hours of exsiccation, became stiff and failed to revive upon replacement in water. Nevertheless they absorbed water at about the same rate and to nearly the same extent as the 2 survivors. The dry weights (dried in oven to constant weight at 100° C.) of these 6 worms averaged 11.935 per cent (range 11.21 to 12.67) of the original fresh weight. The normal water content under the conditions stated was therefore about 88 per cent.

The individual weight curves of these 8 worms appear somewhat variable but roughly logarithmic in form, decreasing rapidly at first and then more slowly. The curves are reversed in form upon reabsorption of water, but the process is slower. It is remarkable that within the first 24 hour period, the weight curves showing loss and gain of the survivors do not differ appreciably from those of the worms killed by the more severe exsiccation. Evidently the limit of tolerance (under the conditions stated) for *Lumbricus terrestris* is a loss of somewhere between 43 and 50 per cent of body weight. For another species of earthworm, *Allolobophora chloroticus*, Hall¹ reported survival after loss of 69.6 per cent of the body weight in 105 minutes of exsiccation. Adolph and Adolph² noted in *Lumbricus terrestris* a loss of body weight up to 50 per cent on placing the worms in salt solutions; also smaller variations in weight according to temperature.

Changes in cross-sectional areas. The remaining 4 earthworms (see Table I) at various stages of exsiccation were anesthetized by chloroform vapor, to obtain uniform relaxation. They were then fixed in 10 per cent formalin solution and embedded in celloidin. The shrinkage by this process is relatively slight. Cross sections of the body just behind the clitellum were stained and mounted. The areas of the entire body and of the constituent layers were enlarged by 2 methods: (1) by photography (x 38), and (2) by outline drawings of the enlarged sections projected on paper (x 103). The various layers were then cut out and weighed, and the corresponding areas calculated. The

¹ Hall, F. G., *Biol. Bull.*, 1922, xlii, 31.

² Adolph, E. F., and Adolph, P. E., *J. Exp. Zool.*, 1925, xliii, 105; *Anat. Rec.*, 1925, xxxi, 340.

two methods give similar results, but the latter appears more accurate.

TABLE I.
Body Weights and Cross-sectional Areas.

Earthworm No.	Original body weight	Final body weight	Length of exsiccation	Loss of body weight	Area of body in cross section
	grams	grams	hours	per cent	mm. ²
12	7.023	7.023	0	0	18.99
10	8.853	6.118	2	30.9	15.08
9	6.742	3.775	4	44.0	13.15
11	7.362	3.885	5	47.2	11.81

As shown in Table 1, the areas (reduced to actual size) for the entire cross sections varied from 18.99 mm.² for the normal control (No. 12) to 11.81 mm.² for extreme exsiccation (No. 11), with loss of 47.2 per cent in body weight. Table II shows the percentages of the total cross-sectional area formed by the areas of the various parts in each of the 4 worms. The *epidermis* appears slightly variable, but in the last case forms a slightly higher percentage of the total area than in the normal. This indicates that the loss in actual area (or volume) for the epidermis is in general nearly proportional to that for the whole body, assuming the cross sections to be typical and the shrinkage uniform in all directions. The same is true for both *circular* and *longitudinal muscle*, excepting the last case, in which the percentages indicate that the muscles have lost relatively less, and therefore appear relatively larger in area. The *intestinal wall*, including typhlosole, large blood vessels and ventral nerve cord, shows similar relations. The *celomic cavity*, including the septa and nephridial tubules, and the *intestinal lumen* remain nearly uniform in relative size (with an exceptional increase in the intestinal lumen of No. 9), up to the extreme case. This is somewhat remarkable, since one might expect the water to be lost from the larger cavities more readily than from the smaller tissue spaces. This appears to occur only in the extreme exsiccation (No. 11), in which the two large cavities are reduced to about half their original relative size.

The individual tissue cells of the various layers cannot be measured with accuracy. In general, however, the cells show surprisingly little change in size or structure. The greatest change is in the epidermis cells, which appear slightly shrunken.

Losses in volume of the various layers. In calculating the volumes of the various layers, the cross-sectional areas were multiplied by the body length. This length was obtained by dividing the body weight by the cross-sectional area of the body (assuming the specific gravity as 1). The losses in volume were calculated for the extreme case, No. 11, in comparison with the normal, No. 12. The volumes in No. 12 were assumed to be the initial values for No. 11, making slight correction for the difference in initial body weight. In this case, with a loss of 47.2 per cent in body weight (or volume), the various layers have apparently decreased in volume by the following percentages: epidermis, -41.0; circular muscle, -35.6; longitudinal muscle, -24.9; intestinal wall, -37.8; celomic cavity, -70.9; intestinal lumen, -76.1.

From the data for the volumes, another calculation was made to show what proportion of the total water loss was derived from each of the various layers, in the extreme case (No. 11). On this basis, the percentages contributed by the various layers appear as follows: epidermis, 5.4; circular muscle, 8.3; longitudinal muscle, 14.0; intestinal wall, 16.3; celomic cavity, 31.5; intestinal lumen, 24.5. Thus the two large cavities apparently contribute 56 per cent, and the muscles 22.3 per cent, of the total water loss in extreme exsiccation. These figures would not be correct for the cases of smaller total loss, however, since in the earlier stages of exsiccation the relative losses are more nearly equal in the various layers, as shown in Table II.

TABLE II.
Percentage of total body area made by each part in cross section.

Earth-worm No.	Epidermis	Circular muscle	Longitudinal muscle	Intestinal wall	Celomic cavity	Intestinal lumen
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
12	6.2	11.0	26.4	20.3	20.9	15.2
10	5.3	11.0	27.0	20.2	22.1	14.4
9	4.8	9.7	27.0	17.5	19.5	21.5
11	6.9	13.3	37.5	23.8	11.6	6.9

SUMMARY.

Lumbricus terrestris, under the conditions stated, survived a loss of 43 per cent in body weight during 5 hours of exsiccation, but did not survive longer periods with losses of 50 to 60 per cent. Nevertheless, the curves of body weight during exsiccation and after replacement in water appear similar in all cases (up to

24 hours). Measurements of cross-sectional areas indicate that during exsiccation in the earlier stages the epidermis, body muscles, celomic cavity, intestinal wall and lumen lose roughly in proportion to their size. But in extreme exsiccation a relatively larger proportion is contributed by the celomic cavity and the intestinal lumen. The loss in the tissues appears chiefly from the intercellular spaces, as the cells (excepting the epidermis) show but slight changes in size and structure.

Pacific Coast Branch

University of California Hospital, February 17, 1926.

3034

Lactic acid in exudates and transudates.

G. D. BARNETT and A. C. McKENNEY, JR.

[From the Medical Division of the Stanford University Medical School, San Francisco, California.]

We have determined lactic acid in a number of serous fluids and in samples of blood taken at the time of paracentesis. Determinations were made immediately by the method of Clausen.

The exudates all showed a considerably greater lactic acid content than that of the blood, the highest values and greatest excess being found in frankly purulent fluids and in sero-fibrinous exudates (streptococcus) which later became purulent. The transudates yielded relatively low figures, usually slightly below the blood values.

The accumulation of lactic acid in inflammatory exudates may be due to bacterial action or to the action of leucocytes on glucose

TABLE I—Exudates.

Case	Clinical diagnosis	Date	Lactic acid in		Excess in Fluid
			Blood	Fluid	
			mg. %	mg. %	mg. %
8	Streptococcus pleurisy	12/16	18	170	152
		12/17	19	165	146
12	Streptococcus pleurisy	1/13	21	148	127
		2/4	23	362	339
16	Empyema		13	318	305
13	Tuberculous pleurisy		15	56	41
10	Tuberculous pleurisy	1/4	23	87	64
		1/14	28	84	56
15	Tuberculous pleurisy		15	97	82
9	Portal cirrhosis and tuberculous peritonitis	12/21	39	60	21
		1/14	93	123	33
2	Carcinoma of pleura		18	43	25

and other metabolites, as noted by Levene and Meyer.¹ In malignant exudates its presence, in concentrations greater than that of the blood, would be explained by the observation of Warburg,² whose study of the respiration of malignant tissues showed a production of lactic acid by cancer cells many times greater than that of normal tissues.

TABLE II—Transudates.

Case	Diagnosis.	Source	Date	Lactic acid in		Excess in Fluid
				Blood	Fluid	
				mg. %	mg. %	mg. %
1	Cardiac cirrhosis	Chest	11/23	15	13	—2
		Abdomen	1/8	22	18	—4
		Chest	2/11	13	25	12
3	Portal cirrhosis	Abdomen	12/8	37	20	—17
		Abdomen	12/14	22	23	1
4	Chronic nephrosis	Chest		17	3	—14
5	Myocardial insufficiency	Chest		20	27	7
7	Myocardial insufficiency	Abdomen		14	21	7
14	Myocardial insufficiency	Abdomen		23	9	—14
17	Myocardial insufficiency	Chest		17	20	3
11	Myocardial insufficiency	Chest		33	24	—9
6	Portal obstruction	Abdomen		33	25	—8

3035

The crystallization of starch.

H. L. VAN DE SANDE-BAKHUYZEN. (Introduced by C. L. Alsberg).

[From the Food Research Institute and the Department of Botany of Stanford University, Stanford University, Calif.]

To 3 grams of wheat starch, ground according to the method of Alsberg and Perry,¹ 100 cc. of distilled water was added at room temperature. This solution was centrifuged for 10 minutes and after removal of the precipitate, centrifuging was repeated twice. To one part of the amylose solution thus obtained two parts of 96 per cent alcohol was added the same afternoon and the

¹ Levene, P. A., and Meyer, G., *J. Biol. Chem.*, 1912, xi, 361; xii, 265.

² Warburg, O., *Wochenschr.*, 1925, iv, 534.

³ Alsberg, C. L., and Perry, E. E., *Proc. Soc. Exp. Biol. and Med.*, 1924, xxii, 60-61.

precipitate with supernatant liquid was allowed to stand for about 3 weeks. In the second week to a part of the precipitate and liquid an equal volume of 96 per cent alcohol was added, giving an alcohol concentration of 80 per cent.

After 3 weeks the precipitates were examined in alcohol under the microscope and were found to contain many spherocrystals. If alcohol is added in excess to the amylose solution a refractive myelin precipitate, consisting of smaller or larger irregular lumps, is formed. This myelin precipitate was later partly present as such but all gradations between amorphous masses and perfect spherocrystals could also be found. The latter consist of separate, very refractive radial needles attached to a central point or nucleus. The needles are about 1μ thick and up to 25μ long. The spherocrystal is formed by several or numerous needles. If the crystal is less perfect, lumps of the less refractive myelin precipitate are still present at its center, so that the needles project only half their length, or the top of the needle is not pointed but formed by a lump that is still amorphous, or the needle itself remains thick and less refractive than the perfect needle, with a more refractive axis in the center. The precipitate from 80 per cent alcohol showed more numerous and more perfect spherocrystals than the precipitate from 64 per cent alcohol.

With alcoholic iodine, the spherocrystals are colored brown to purple. It is suggested that the slow formation of the crystals is a continued dehydration process, as discussed in a previous paper.² The perfect crystals are those that are most dehydrated.

Under the polarizing microscope with crossed nicols, the spherocrystals were luminous, the needles appearing separately. A black cross could not be observed, probably because the needles are not numerous enough in all directions. If both nicols are rotated at the same time, the needles show parallel extinction.

In a previous paper³ it was pointed out that the starch grains are built up of radial needles. The formation and behavior of the spherocrystals, arising from the precipitate which is formed if alcohol is added to amylose solution at room temperature, agree very well with these observations.

² PROC. SOC. EXP. BIOL. AND MED., 1925, xxiii, 195-197.

³ *Ibid.*, 1926, 302-305.

VII. Active principles of tuberculin prepared from non-protein substrates.

FREDERICK EBERSON

[From the Department of Medicine, University of California, San Francisco, Calif.]

Diagnostic tuberculins which are used routinely at the present time, appear to be unsatisfactory because they lack certain requirements. The ideal preparation should be simple and constant in composition and should be prepared by a standard method; it should be free from substances which give non-specific reactions and these should show reasonable correlation with the extent and severity of the tuberculous infection. Because the interpretation of a tuberculin test depends chiefly upon what is seen on the skin, it is important that there be no extraneous factors modifying the capacity of the skin to react specifically. Since vasomotor excitability appears to run parallel with the intensity of the tuberculin reaction, it is evident that non-specific proteins or indeterminate irritating substances which affect vasomotor excitability should be excluded.

For these reasons the problem has been studied by using tuberculins prepared from non-protein synthetic media. Preliminary studies with such material were reported by the writer.^{1, 2} These tuberculins contain only elements manufactured by the tubercle bacilli in the nutrient substrate. From this media it is possible to separate chemically a number of fractions which represent a tuberculin principle more accurately than ordinary complex tuberculins.

The present investigation includes (A) sensitization experiments in normal, non-tuberculous guinea pigs, (B) intracutaneous tests in tuberculous guinea pigs, and (C) clinical diagnostic tests with tuberculin fractions in a series of juvenile patients.

Preparation of Tuberculin Fractions. Tuberculin was prepared from non-protein media of the following composition, according to a formula of Gessard and Vaudremer:³

¹ Ebersson, F., *Proc. Soc. Exp. Biol. and Med.*, 1925, **xxii**, 346.

² Ebersson, F., *Ibid.*, 1924, **xxi**, 539; *Am. Rev. of Tuber.*, 1925, **x**, 681; *Am. J. Dis. Child.*, 1925, **xxix**, 29.

³ Gessard, C., and Vaudremer, A., *Compt. rend. Soc. de biol.*, 1922, **xxcvi**, 1012.

Ammonium succinate	0.5	gm.
Dipotassium phosphate	0.5	gm.
Magnesium sulphate	0.25	gm.
Calcium chloride	0.125	gm.
Distilled water	100.0	cc.

To one portion of this substrate 2 per cent glycerine was added. After the tubercle bacilli had been growing on this media for five to six weeks, the tuberculin was prepared according to the usual method by filtration and concentration to one-tenth of the original volume. The growth and staining properties of tubercle bacilli were modified by this synthetic media. In the glycerinated portion the organisms grew more luxuriantly than in the portion to which no glycerine had been added. In the former, half of the organisms lost their original acid-fast characteristic, while all of them lost it in the glycerine-free media.

A weighed volume of tuberculin (100 gm.) was fractionated by adding it to 19 volumes of pure methyl alcohol to effect complete precipitation. The filtrate, after treatment with four volumes of pure ethyl ether, yielded a precipitate containing a second fraction. From the resultant filtrate a third fraction was obtained by concentrating the material at 37 degrees Centigrade to practical dryness. These substances were dissolved separately in a given volume of physiological salt solution to which 0.25 per cent ether-tricresol was added as a preservative. The alcohol-insoluble fraction represented 4.6 per cent by weight of the original tuberculin, and one cubic centimeter of the test material contained 49.8 milligrams of active substance. The ether-insoluble fraction, 2.3 per cent by weight of the original tuberculin, contained 25.3 milligrams of active substance in one cubic centimeter. The ether-soluble third fraction was exceedingly small in amount and up to the present time has not been checked with sufficient quantitative accuracy to warrant a definite figure. Roughly, it represented less than 0.5 per cent by weight of the original tuberculin and contained approximately 4.4 milligrams of active substance in one cubic centimeter. This fraction was gummy and fatty or waxy and *gave none of the tests for protein.*

Sensitization Experiments in Normal, Non-Tuberculous Guinea Pigs. Duplicate series of normal guinea pigs weighing 250-300 grams were injected weekly with gradually increasing amounts of each of the tuberculin fractions by the subcutaneous route. The number of injections totalled ten and ranged from

0.1 cubic centimeter for the first dose to 1.2 cubic centimeters of the different fractions at the time of the last injection. From the quantitative determinations it was calculated that the animals received the following total amounts of active substances in the course of treatment: Alcohol-insoluble fraction, 318.7 mg.; ether-insoluble fraction, 192.3 mg.; ether-soluble fraction, 29.5 mg.

Intracutaneous tests were made one week after the last injection. Homologous as well as heterologous fractions were injected in amounts of 0.01 cubic centimeter into the shaved and sterilized skin of the abdomen. The results were read after 24 hours and daily thereafter. The outcome of these experiments was decisive and confirmed the studies which have been alluded to already. Of added significance here is the fact that *protein-free substrates yielded a tuberculin which was active in toto and contained specific sensitizing substances that could be separated out by chemical methods*. One or more injections of derivatives or fractions from ordinary tuberculin or from a non-protein tuberculin substrate, therefore, sufficed to elicit typical skin reactions in non-tuberculous guinea pigs. These results are illustrated in Figures 1-3. Control animals were uniformly negative with the same substances.

Intracutaneous Tests with Tuberculin Fractions in Tuberculous Guinea Pigs. In the tuberculized guinea pig it was demonstrated that these same fractions would give unequivocally positive skin reactions by the intracutaneous technic. The results were obtained repeatedly in several duplicate series of animals. Such tests were made after the inguinal lymph nodes had become palpable in animals previously inoculated subcutaneously with a constant amount of human tubercle bacilli of fixed virulence (1.2 mg. dry weight). In most instances this occurred after three weeks. Each of the test substances was injected in amounts of 0.01 cubic centimeter into the skin of the abdomen, and the results were noted after 24, 48, and 72 hours. An illustration of such a reaction with the ether-insoluble fraction is shown in Figure 4.

Clinical Diagnostic Tests with Tuberculin Fractions in Children. Intracutaneous tests have been made thus far on 150 children and a smaller group of adults. Sixty juvenile patients have been tested with fractions prepared from ordinary tuberculin.

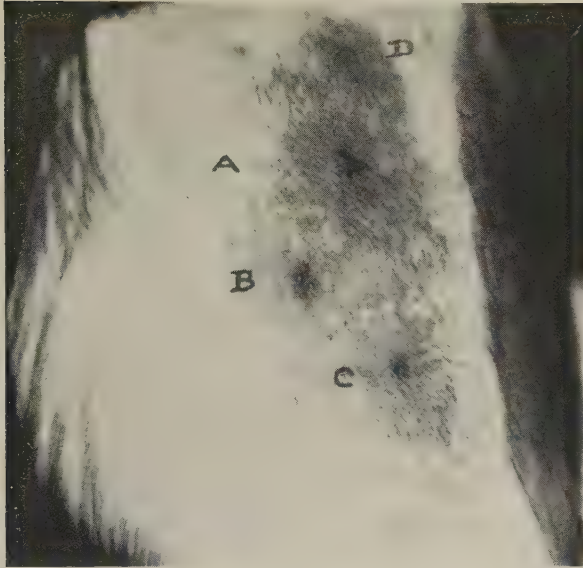


FIG. 1.

Intracutaneous tests in non-tuberculous guinea pig sensitized with ether-soluble fraction prepared from synthetic, protein-free tuberculin substrate, non-glycerinated. Reactions 24 hours old to 0.01 cc. of homologous fraction, (D); ether-soluble fraction (glycerinated substrate), 0.01 cc., (A); ether-insol. fraction (glycerinated substrate), 0.01 cc., (B); original tuberculin from protein-free, glycerinated substrate, 0.01 cc., (C).

The remainder received protein-free substrate tuberculin and its fractions. These preparations, eight in number, included a duplicate series of substances which were derived from glycerine-free tuberculin substrates. Controls were made with ordinary human and bovine tuberculins and with physiological salt solution in each patient. For the intracutaneous tests 0.1 cubic centimeter of a 1:1,000 dilution of each test substance was injected. The results were read after 24, 48, and 72 hours. The amount of "tuberculin substance" which was injected differed for each preparation. The fractions contained decidedly less of the active substance in any give dose in comparison with ordinary tuberculin, yet they proved to be potent as well as specific. A few of these reactions are illustrated in Figures 5-7. The relative amount of active substance in a given test was calculated as follows: Ordinary old tuberculin, 0.1 mg.; alcohol-insoluble fraction, 0.005 mg.; ether-insoluble fraction, 0.0025 mg.; ether-soluble fraction, 0.0005 mg.



FIG. 2.

Intracutaneous tests with 0.01 cc. of tuberculin fractions in non-tuberculous guinea pig sensitized with ether-insoluble fraction prepared from synthetic, protein-free tuberculin substrate, glycerinated. Reactions 24 hours old. Homologous fraction, ether-insoluble, (A); ether-soluble fraction (glycerinated substrate), (B); original tuberculin from glycerinated substrate, (C).

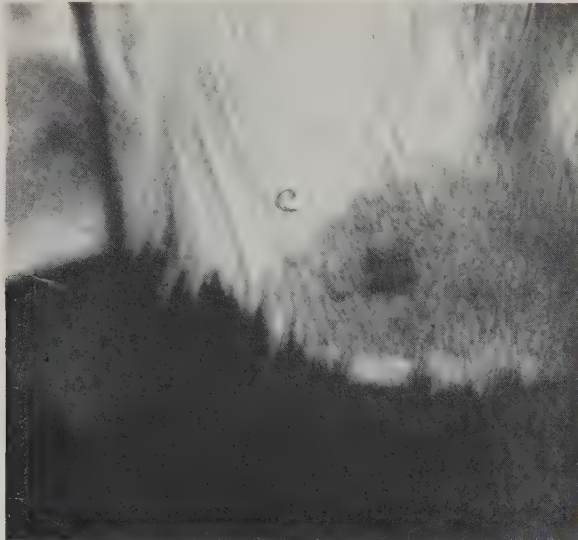


FIG. 3.

Same guinea pig as in Fig. 2, showing reaction in natural size at (C), corresponding to intracutaneous injection with 0.01 cc. of tuberculin from glycerinated non-protein substrate.

Intracutaneous tests with tuberculin fractions gave definite skin reactions which were usually less severe than those which were observed with ordinary tuberculins. The results, nevertheless,

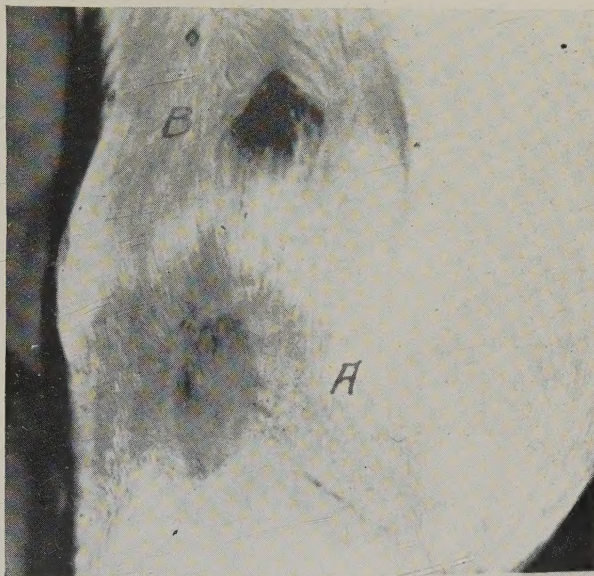


FIG. 4.

Tuberculous guinea pig, three weeks after inoculation with human tubercle bacilli. Intracutaneous test with 0.01 cc. ether-insoluble fraction derived from glycerinated non-protein tuberculin substrate. (A) drainage tuberculous abscess at (B). Skin test 24 hours old.

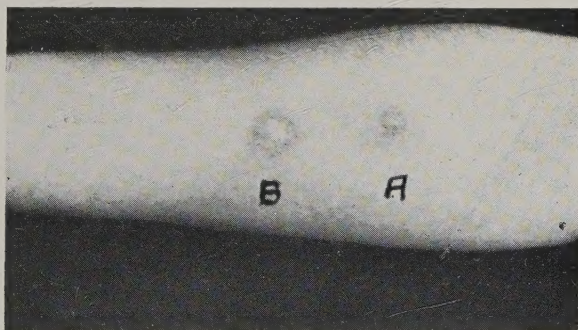


FIG. 5.

Intracutaneous tests in patient with peribronchial tuberculosis and tuberculosis of cervical lymph nodes. (A) 0.1 cc. of 1:1,000 dilution of ether-insoluble fraction from non-protein, glycerine-free tuberculin substrate. (B) 0.1 cc. of 1:1,000 dilution of same fraction with glycerinated substrate. Amount of active substance in fraction, 0.0025 milligram. Reaction 24 hours old.

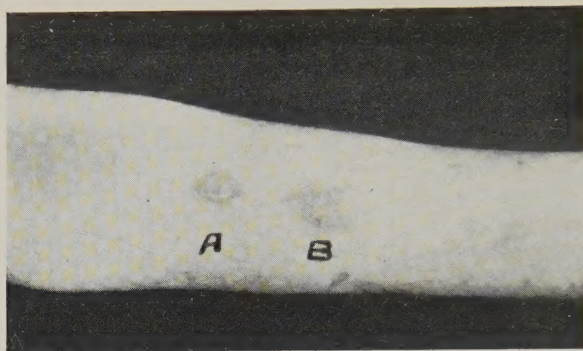


FIG. 6.

Intracutaneous tests in patient with peribronchial tuberculosis. (A) 0.1 cc. of 1:1,000 dilution of non-protein, glycerine-free tuberculin. (B) 0.1 cc. of 1:1,000 dilution of same substance, glycerinated. Amount of active tuberculin substance, 0.008 milligram. Reaction 48 hours old.

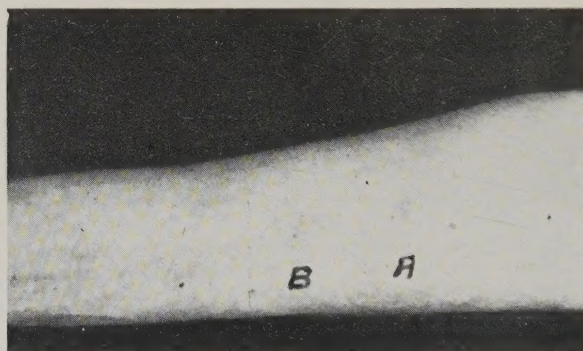


FIG. 7.

Intracutaneous tests in same patient as in Fig. 6. (A) 0.1 cc. of 1:1,000 dilution of ether-soluble fraction from non-protein, glycerine-free tuberculin substrate. (B) 0.1 cc. of 1:1,000 dilution of same fraction from glycerinated substrate. Amount of active tuberculin substance, 0.0005 milligram. Reaction 72 hours old.

could be better correlated with the clinical histories, physical findings, and with the X-ray. In certain instances the observations suggested that the reactions might be correlated with the degree of activity or of an arrested condition in a given tuberculous infection. However, more data are being analyzed in order to verify this striking observation. Of special interest was a group of contact patients in a number of whom a clinical diagnosis of tuberculosis could not be made. In these children the ordinary tuberculins gave markedly positive reactions while the fractional tuberculins gave negative reactions. This was true

also in another group of patients who had no positive clinical findings and whose X-ray films showed bronchial adenitis.

Summary of Tuberculin Tests in a Series of 150 Children.

	Per cent
Positive intracutaneous tests with ordinary O. T. and with tuberculin fractions -----	70.0
Positive tests with ordinary O. T. and negative tests with tuberculin fractions -----	30.0
Positive tests with ordinary O. T. in patients with negative clinical findings and negative X-ray -----	22.0
Positive tests with tuberculin fractions in patients with negative clinical findings and negative X-ray -----	00.0

SUMMARY AND CONCLUSIONS

It has been shown for the first time that small amounts of tuberculin fractions prepared from protein-free synthetic substrates are capable of sensitizing normal, non-tuberculous guinea pigs. In these animals, typical skin reactions can be elicited subsequently by intracutaneous injection of minute amounts of homologous as well as heterologous fractions and of unfractionated tuberculin from which such fractions have been prepared.

Clinical trial of these fractions in juvenile patients has demonstrated for the first time that the substances have diagnostic value. Positive skin tests were perfectly correlated with positive clinical and laboratory findings of tuberculous infection. Such was not the case for ordinary old tuberculin in over 20 per cent of young patients in a group of 150 who were studied routinely. In a number of instances the observations suggested that the reactions might be correlated with the degree of activity or with arrested tuberculous infection.

The present studies point to a method which makes an accurately standardized tuberculin available for routine clinical work. It is believed that the active principle of tuberculin can best be isolated by this or by a similar method.

The observations on sensitization suggest that these may be applied to studies of immunity, and by extension, to therapeutic studies in the experimental animal. Such work, now in its third year of progress, will be reported in the future.

